Objective Assessments of Pruritus in Children with Atopic Dermatitis

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A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree of

Master of Philosophy

in

Medical Sciences

June 2006

ACKNOWLEDGEMENTS

It is of my greatest eagerness to deliver my heartful thanks with gratitude to my supervisors, Professor Ellis Hon and Professor Ting-Fan Leung of the Department of Paediatrics, The Chinese University of Hong Kong, for their profound and continuous teachings, guidance, support, care and freedom given to me throughout the study over the past two years. Without their invaluable comments, the compilation of the thesis would not be made into a completion.

Heartfelt gratitude is expressed to Miss Carmen Fong, manager of the Institute of Chinese Medicine, the Chinese University of Hong Kong, and respective staff, for their expertise advice and management in Traditional Chinese Medication clinical trials that helped me a lot throughout.

I appreciate Miss Brenda Chung-Yi Li for her assistance in laboratory techniques, Miss Christy Wai-Yan Kam and Miss Kin-Yee Wong for co-coordinating the studies with me, and Mr Edmund Yung for his kind help in many aspects.

Additional thanks must go to every research team member and buddy of the Department of Paediatrics, for their technical and moral support. Without any one of them, the project would not be accomplished in such a harmonious and smooth manner.

Last but not least, may all of my sincere gratitude and praise flow towards my beloved family members, soulmate, and the Lord God our Father.

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Abstract:

Atopic dermatitis (AD) is a chronic, distressing disease, affecting up to 20% of Hong Kong child population. It is associated with pruritus and sleep disturbance. The sensation of pruritus involves a complex neuro-signaling pathway. Scratching due to itching is an important mechanism in the pathogenesis of AD but is difficult to be documented. Subjective symptoms of pruritus and sleep loss reported by parents correlated only weakly with the objective signs (extent and intensity) of AD severity, whereas traditional objective assessments are inconvenient and cannot be carried out in a home-based environment.

A new wristwatch-alike motionlogger has therefore been adopted as a trial for home-based convenient assessment of itch. Forty-three Chinese children with AD (mean [SD] age of 11.9 [3.6] years) and thirty-seven normal children, aged 11.9 (3.4) years were recruited for device validation. 2 clinical trials of traditional Chinese herbal medication and Tacrolimus calcineurin inhibitor were also conducted. AD disease severities of the subjects were firstly evaluated with the SCORing Atopic Dermatitis (SCORAD) index. Subjective reports of pruritus, sleep loss and a questionnaire of Children's Dermatology Life Quality Index (CDLQI) were performed. Concentrations of plasma AD-associated chemokines (cutaneous T cell attracting cytokine, CTACK; thymus and activation regulated chemokine, TARC) and

pruritus-causing laboratory markers (Brain-derived neurotrophic factor, BDNF and substance-P) were measured in patients. Patients were then instructed to wear Digitrac® movement recorder (IM Systems, Baltimore, MD) on their dominant wrist before sleeping. The monitor had been programmed to start logging limb motion continuously from 22:00 to 08:00 h the next morning. The contour pattern between eczema patients and controls were compared to see if any unique patterns could be distinguished.

When visually compared with controls, most wrist activities occurred at frequencies from 0 to 3 hertz. These activities were most consistent over the first three hours of sleeping and significantly correlated with disease extent, intensity, plasma AD-associated chemokines and pruritic markers. However, there was no significant correlation between wrist activities and subjective scorings. Wrist activity measurement by such a device is therefore useful in obtaining the pruritus and sleeping data of patients conveniently in an objective manner, and leaves a large room for applying to determine drug efficacies of various AD medications and other diseases associated with pruritus.

論文概要:

異位性皮膚炎是一種慢性及困擾的病症。全港大約有約百分之二十的兒童正受到該症困擾，而該病症往往與痕癢和睡眠干擾聯繫在一起。人體對痕癢的感覺牽涉一個很複雜的神經信號網絡，而抓癢雖然是一種異位性皮膚炎的重要病發原理機制，但很難被證明。另一方面，病人對痕癢及睡眠影響的主觀匯報與客觀的疾病程度、強度標示只有微弱的關聯，而同時傳統的客觀評估方法十分不方便，並不能在家中環境進行。

因此，一個嶄新的手部運動紀錄儀被正式用在是次測試中，以在家中環境作客觀評估痕癢程度之用。四十三名患有異位性皮膚炎病童及三十七名對照參加者被邀請作是次儀器檢驗之用，另外兩個中草藥及 Tacrolimus 鈣調神經磷酸酶抑制劑之臨床測試亦有進行。病童的病症嚴重程度以異位性皮膚炎評分指標(SCORAD)作評估，而病人對痕癢及睡眠狀況的主觀報告及兒童皮膚科生活質素指數問卷亦同時進行。病童血漿內與異位性皮膚炎有關聯之胸腺和活化調節趨化因子(TARC)、皮膚 T 细胞吸引趨化因子(CTACK)，及與痕癢有關聯之腦衍生神經滋養因子(BDNF)和 P 物資(SP)之濃度亦被量度作研究用。病童會按指示在睡眠前配戴 Digitrac® 手部運動紀錄儀在主手腕上，該儀器被設定連續記錄由晚上十時至翌日早上八時的手部活動情況。病童與對照參加者的活動分佈輪廓會作比較，藉以觀察有否特別活動模式。

當通過程式與對照比較，病童多數手腕活動發生頻率位於 0 到 3 赫茲內，

而這些活動在頭三小時的睡眠是最一致的，並與疾病程度、強度、血漿內與濕疹有關之趨化因子及痕癢指標有顯著關聯。不過，手腕活動與主觀評分沒有顯著關聯。因此，利用該儀器能有效並客觀地獲取病人的痕癢和睡眠數據，並在應用於評估濕疹治療藥物功效及其他與痕癢有關聯之病症存在很大空間。

List of abbreviations (in order of occurrence)

AD	Atopic dermatitis
APCs	Antigen-presenting cells
BDNF	Brain-derived neurotrophic factor
C3	Complement-3
CC	Conserved cysteines
CDLQI	Children's Dermatology Life Quality Index
CLA^+	Cutaneous lymphocyte antigen-positive
CS	Corticosteroids
CTACK	Cutaneous T-cell attracting chemokine
ELISA	Enzyme-linked immunosorbent assay
FcεRI	High-affinity receptor for IgE
FDA	Food and Drug Administration
FKBP	FK-binding protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
Hz	Hertz
IDEC	Inflammatory dendritic epidermal cells
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IL-4,/IL-5,IL-13	Interleukins 4,5,13
KMO	Kaiser-Meyer-Olkin
NF-AT	Nuclear factor for activated T-cells
PAR-2	Proteinase-activated receptors 2
PB	Peripheral blood
PG	Pemphigoid Gestationis
PSG	Polysomnography
QoL	Quality of life
S. aureus	*Staphylococcus aureus*
SCORAD	SCORing Atopic Dermatitis index
SD	Standard deviation
SEM	Standard error of mean
SP	Substance P
SPSS	Statistical Package of Social Sciences
TARC	Thymus and activation-regulated chemokine
TCHM/TCM	Traditional Chinese Herbal Medication
T_H0	T-helper cells

T_H1	T-helper 1 cells
T_H2	T-helper 2 cells
TIMs	Topical immunomodulators
TNF-α	Tumor necrosis factor –α
UVA	Ultraviolet A

Publications

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   CDLQI, Scorad and Ness: Are they Correlated?
   Qual Life Res. 2006 Jul 7.

2. Hon KL, Lam MC, Leung TF, Kam WY, Lee KC, Li MC, Fok TF, Ng PC.
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Chapter 1

Introduction and Literature Review

1.1 General aspects of atopic dermatitis

1.1.1 Definition and its nature

Atopic dermatitis (AD) (or atopic eczema) is one of the most common chronic inflammatory skin disorders occurring in infants and children. The term 'atopy' was first being used by two allergists, Coco and Cooke, and the term 'atopic dermatitis' was originated in 1933 in order to distinguish it from other dermatologic disorders (Figure 1.1) (Coco and Cooke, 1923;Williams *et al.*, 1994). Atopy has been defined by the European Academy of Allergy and Clinical Immunology (EAACI) Nomenclature Task Force as "a personal tendency, familial tendency, or both, usually in childhood and adolescence, to become sensitized and produce immunoglobulin E (IgE) antibodies" (Johansson *et al.*, 2001). The World Allergy Organization has also recently published its recommended terminology (Flohr *et al.*, 2004). There is no universal agreement about the definition of AD at present. Some prefer to define its atopic nature using IgE-specific antibodies and peripheral eosinophilia, which will develop in response to allergen exposure (Bos, 2002;Turner, 2006;Boguniewicz and Leung, 2006); while others believe that AD defines a clinical state with characteristic criteria and features (Hanifin and Rajka, 1980;Williams *et al.*, 1994).

1.1.2 Epidemiology of atopic dermatitis

The cardinal skin features of atopic dermatitis are erythematous and eczematous lesional skins, generalized dry skin, flexural lichenifications and papules accompanied by

intense pruritus and cutaneous hyperactivity (Kay, 2001). Most often, edematous lesions appeared on the skin surface during the acute phase, often excoriated with superficial crusts. When AD enters a chronic phase, the skin possess a complex phenotype with lichenified plaques, skin thickening and scaling, accentuated skin markings and post-inflammatory hyper- or hypopigmentation. In paediatric patients, lesions are more common at the flexural joints, neck, forehead, cheeks, wrists and dorsa of limbs (Fleischer *et al.*, 2002). Up to now, 2 types of AD have been identified: extrinsic AD, affecting 70-80% of AD patients, presenting with sensitization towards environmental allergen and increased serum IgE levels; and the intrinsic form of AD, affecting 20-30% of the patients accompanied by low IgE levels and absence of any detectable allergen sensitization (Schmid-Grendelmeier *et al.*, 2001;Novak *et al.*, 2003).

The prevalence of AD in children is estimated at about 15%, which is a 3- to 4-fold increase when compared with the trend in 1950s (Lewis-Jones, 2001) and is equally common in many other countries worldwide (Leung *et al.*, 1997;Foley *et al.*, 2001). It develops early in life and becomes clinically evident in approximately 90% of children before 4 years of age (Emerson *et al.*, 1998). It is highly pruritic, chronic and is commonly present during early infancy and childhood. However, the disease can persist or start in adulthood (Johansson and Bieber, 2002). Several longitudinal studies suggest that AD predisposes to the development of allergic rhinitis and asthma and together these 3 allergic disorders are often being recognized as "atopic triads", often with AD initializing the atopic march (Boguniewicz *et al.*, 2003;Leung *et al.*, 2004;Spergel, 2005).

1.1.3 Factors and triggers related to high risk of atopic dermatitis

Genetics

It has been proved that atopic dermatitis is genetically complex with strong maternal influence, with parental AD conferring a higher risk to offspring than parental asthma or allergic rhinitis, suggesting presence of AD-specific susceptibility genes (Cookson and Moffatt, 2002;Cookson, 2004;Brown and Reynolds, 2006). Different candidate genes have already been intensely investigated basing on their theoretical roles in pathogenesis of AD, including those lying in chromosome region 1q21 showing genes specific for AD (Sugiura *et al.*, 2005), 3q21 for CD 80 and 86 (Lee *et al.*, 2000), 5q31-33 for interleukin-13 (Hummelshoj *et al.*, 2003), 11q13 for β-subunit of high-affinity receptor for IgE (FcεRI) (Folster-Holst *et al.*, 1998) and 17q25 for different gene and protein expressions between asthma and AD patients (Cookson *et al.*, 2001;Ekelund *et al.*, 2006), etc. However, a recent study in Chinese Population showed that there are not any associations of gene polymorphism patterns with expression of various cytokine levels (Chang *et al.*, 2006). Nevertheless, there is still a long way to go to reach a consensus about the particular gene or genes that plays a key role in AD.

Allergens

Only the genetic factor is not able to give the whole picture for the causes and difference of atopic dermatitis (Williams, 2005). Most often, AD is also immunologically triggered by various environmental factors and allergens, for example, food allergens (Hon *et al.*, 2006b). Food allergens would induce skin rashes in a significant proportion of children with moderate to severe AD (Sampson, 1999), and they would have an

elevated food-specific IgE levels with immediate positive skin test results (Lever *et al.*, 1998). Yet the dietary practices in different regions are different, thus showing a wide range of food allergen potency for different races of paediatric patients (Hon *et al.*, 2006b).

Other common types of allergens include aeroallergens, which might cause pruritus and lesions after bronchial or intranasal inhalation (Tupker *et al.*, 1996); and autoallergens, which the IgE or T-cells inside their own hosts will attack against, thus leading to immunological responses(Valenta *et al.*, 2000;Kinaciyan *et al.*, 2002).

Staphylococcus aureus (S. aureus)

Bacterial superinfection is common in children with atopic dermatitis (Ong *et al.*, 2002). It was reported that over 90% of the skin of the AD patients were colonized with *S. aureus* (Leyden *et al.*, 1974), and that a combination of treatment with both antibiotics and corticosteroids is more effective than corticosteroid therapy alone (Leyden and Kligman, 1977), suggesting the importance of such a bacterial infection. Colonization of *S. aureus* is important to the pathogenesis of AD. The bacterium produces superantigens to activate T-cell and macrophages to release cytokine, cause mast cell degranulation and enhance IgE-mediated reactions, thus influencing disease activity (Bunikowski *et al.*, 2000;Hon *et al.*, 2005a).

To summarize, the clinical phenotype of atopic dermatitis is determined by a complex interplay of the patients' susceptibility genes, their environment, skin barrier defects and various immune responses (Leung *et al.*, 2004).

1.2 Pathogenesis of atopic dermatitis

1.2.1 Nature of complexity of pathogenesis

Although characteristic phenotypes make AD diagnosis to be simple and clear-cut (Barnetson and Rogers, 2002), its underlying pathophysiology mechanisms are very complex and debatable, and still not completely understood, although various cell types (e.g. lymphocytes, Langerhans cells, eosinophils, keratinocytes) and factors (e.g. cytokines, chemokines and immunoglobulins, particularly IgE) have been identified (Friedmann, 2002).

1.2.2 Role of T-helper cell in atopic dermatitis and its paradigm model

A commonly accepted immunologic pathway of AD pathogenesis is the T-helper 1 cell / T-helper 2 cell (T_H1/T_H2) paradigm model (Abramovits, 2005). It starts from the differentiation of CD_4^+ T-helper lymphocytes (T_H0) into T_H1 and T_H2 cells. In AD patients, T_H2 imbalance over T_H1 is prevailed and enhanced. Obsessive amount of T_H2 lymphocytes produce interleukins 4, 5 and 13 (IL-4, IL-5, IL-13), which provide signals for B lymphocytes to activate mast cell, produce IgE and eosinophils, thus promoting type-1 hypersensitivity reactions in acute phase of AD (Jelinek, 2000;Friedmann, 2002;Liu *et al.*, 2006) (Figure 1.2). T_H1 lymphocytes produce IL-2 and interferon gamma (IFN-γ), provoking delayed hypersensitivity reactions (Knoell and Greer, 1999). The two T-cells are mutually affected in the way that cytokines produced by T_H2 suppress T_H1 activities (Abramovits, 2005), whereas IFN-γ produced by T_H1 suppressed T_H2 (Grewe *et al.*, 1998) (Figure 1.2). Nevertheless, along the whole course of AD development from acute to chronic phase, the patients exhibit a biphasic T-cell pattern in which T_H2

immune response predominance will switch to a more T_H1-like profile respectively (Leung, 2000).

1.2.3 Nature of immunoglobulin-E and its role in atopic dermatitis

Basing on the above-proposed model, the majority of AD patients will therefore exhibit a hyper-production of immunoglobulin E as a result of the enhanced production of T_H2 cytokines, particularly during the onset or the acute lesional phase (Abramovits, 2005). Another source of IgE production is due to presence of *Staphylococcus aureus* antigens (Nordvall *et al.*, 1992) and other superantigens (Marrack *et al.*, 1990). It has been documented for long that IgE is the key mediator of AD and related hypersensitivities towards environmental allergens (Church *et al.*, 1976), and a hallmark of atopic diseases (Burrows *et al.*, 1989). Recent studies continue to prove that total serum IgE levels have significant epidemiological associations with early AD incidence, its subsequent prognosis (Perkin *et al.*, 2004) and its severity (Laske and Niggemann, 2004). Nevertheless, it has to be reminded that the above definition did not apply to the group of intrinsic form of AD patients, who exhibit normal values of total or specific IgE (Dotterud *et al.*, 1995).

IgE in peripheral blood forms crosslinks with antigens and triggers type I hypersensitivity reaction by inducing mast cells and basophils to release mediators, including histamines, which are the main pruritus-causing suspects (Abramovits, 2005). IgE also poses its significance on antigen-presenting cells (APCs) (Langeveld-Wildschut *et al.*, 2000). The number of IgE-bearing Langerhans cells and inflammatory dendritic epidermal cells (IDEC) expressing FcεRI also increases (Novak *et al.*, 2001). Langerhans

cells provide a positive feedback loop by increasing the pool of T_H2 cells, while FcεRI capture and internalize incoming antigens before they are processed to T lymphocyte in atopic skins (Santamaria-Babi, 2006).

1.2.4 Chemokines in pathogenesis of atopic dermatitis: CTACK and TARC

Another recent approach of the understanding towards the disease is by looking at relating chemokines. Chemokines are small, secreted polypeptides, regulating infiltration and migration of inflammatory lymphocytes into tissues through G-protein coupled 7-transmembrane domains (Hijnen *et al.*, 2004). They are pro-inflammatory cytokines, released by different cell types to function as chemoattractants and to guide cells involved in innate immunity. In AD, chemokines are responsible for guiding cutaneous lymphoid antigen T cells to the dermis (Morales *et al.*, 1999). Their importance is substantiated by the fact that they express more granulocyte-macrophage colony-stimulating factor (GM-CSF) (Pastore *et al.*, 1997). GM-CSF initiates and maintains the chronic inflammatory process of AD by inducing generation, maturation and enhancement of APCs (Girolomoni and Pastore, 2001) and is therefore a central feature of AD.

Among all chemokines, the thymus and activation-regulated chemokine (TARC) and cutaneous T-cell attracting chemokine (CTACK) are suspected to be important in AD pathogenesis and were shown to be specific for AD, which will increase with AD severity and decrease with successful treatment (Hijnen *et al.*, 2004). Extensive studies on the 2 chemokines these few years further confirm their leading roles as biological markers for clinical management of the disease (Boguniewicz and Leung, 2006). The

receptor of TARC is richly expressed on skin homing, cutaneous lymphocyte antigen-positive (CLA^+) T lymphocytes (Campbell *et al.*, 1999). It is shown that TARC induces migration of T_H2 cells (Imai *et al.*, 1999), triggering a cascade of immune responses to tackle against allergic disorders. Such a theory is proved when TARC levels are found to be elevated in patients with AD, comparing with patients with psoriasis and healthy control subjects (Kakinuma *et al.*, 2001).

However TARC might nor be skin-specific and might be altered by other concurrent atopic disorders (Leung *et al.*, 2002). CTACK is a skin-specific chemokine, shown to be useful in accessing adult AD severities (Kakinuma *et al.*, 2003;Hon *et al.*, 2004a). It is a member of the conserved cysteines (CC) chemokine family, in the form of a ligand for CC chemokine receptor 10 (Kunkel and Butcher, 2002) and is responsible for localization of CLA^+ memory T cells to skin, thus regulating T-cell trafficking in cutaneous levels (Morales *et al.*, 1999).

1.2.5 Role of antimicrobial peptides and innate immunity

Antimicrobial peptides (AMP) are an integral part in mediating human innate defense. They are expressed by epithelial or adjacent cells, being secreted onto skin surface in response to inflammation and perform antimicrobial activity against microorganisms, including *Staphylococcus aureus* (Ong *et al.*, 2002). Classes of AMP include human cathelicidin LL-37, human β-defensin classes and others (Gallo and Huttner, 1998). In AD patients, the innate immune mechanism is impaired. Low levels of AMP will therefore promote colonization of *S. aureus* on nonlesional atopic skin, and worsen AD (Rieg *et al.*, 2005).

1.3 Measurements of atopic dermatitis severity and quality of life impairment

1.3.1 Scoring of atopic dermatitis severity and the SCORing Atopic Dermatitis Index

Despite the current ambiguities in understanding of AD, clinicians and researchers are seeking for the most valid, reliable, and relevant outcome measures to ensure the effectiveness of healthcare interventions (Charman *et al.*, 2003).

Due to the fact that AD is a syndrome of related clinical features arising in response to a number of endogenous and exogenous factors, there is not a sole definite marker to reflect definite severity of eczema. Therefore measurements are mainly based on a collection of signs and symptoms (Finlay, 1996). A review of 93 randomized controlled AD-related clinical trials revealed that scoring of clinical signs was the most widely adapted outcome measure (in 91% of trials) (Charman *et al.*, 2003). According to the review, the most widely used clinical scoring system was the SCORing Atopic Dermatitis (SCORAD) index (1993), a score basing on a set of 24 major and minor clinical diagnostic criteria of AD proposed by Hanifin and Rajka (Hanifin and Rajka, 1980) (Table 1.1). In SCORAD, 60% of the total score was based on clinical signs and 20% is based on disease extent, with only 20% weighting for patient reporting subjective symptoms of pruritus and sleep loss (Kunz *et al.*, 1997;Gelmetti and Colonna, 2004).

1.3.2 Quality of life measurement

While a lot has been discussed on scoring of AD, quality of life (QoL) has always been neglected, with only 3% of reviewed clinical trials had proper measurements (Charman *et al.*, 2003). The impact of life quality impairment of AD is a very important manifestation as it has both a high rate of prevalence and is often more severe and long-lasting than any other childhood skin disorders (Finlay and Burgdorf, 2004). It can cause

severe psychological problems and can be overwhelming not only to the patients, but the entire family (Barnetson and Rogers, 2002). Moreover, SCORAD has its own shortcomings. Although SCORAD is one the best validated systems and is suited for clinical trials, it is too complicated and time consuming for routine clinical use (Gelmetti and Colonna, 2004). In patients' point of view, an objective clinical scoring system, like SCORAD, is also not necessary to provide a clinically all-rounded meaningful reflection of disease severity, with SCORAD focusing QoL impairment on pruritus and sleep loss only (Charman *et al.*, 2005). Therefore patient-based symptom measures and QoL scoring systems have also been emphasized over the past decade for providence of additional information (Jordan, 1983;Lindstrom and Kohler, 1991;Lewis-Jones and Finlay, 1995;Holm *et al.*, 2006).

1.3.3 The Children's Dermatology Life Quality Index (CDLQI)

Among all, the Children's Dermatology Life Quality Index (CDLQI) is one of the most standardized and objective QoL measures (Lewis-Jones and Finlay, 1995). It consists of 10 questions, designing for use in children ages 5-16 years. It is self-explanatory and can be filled up by the child with minimum help from the parent or caretaker. Questions in the index cover over the areas of symptoms and feelings, daily activities, leisure, impact during school or on holiday, personal relationships, and sleep and treatment compliance. The CDLQI has been validated and translated into Cantonese to suit local clinical and community epidemiology usage in Hong Kong (Chuh, 2003).

1.4 Pruritus in atopic dermatitis and its underlying mechanisms

1.4.1 Introduction to pruritus

Pruritus, or itch, has for long been defined as a poorly localized, non-adapting, usually unpleasant sensation that provokes desire to scratch (Rothman, 1941) and is a major important symptom of atopic dermatitis, constituting a predominant diagnostic criterion. It is one of the most complex and multi-dimensional sensations, and its research progress is slow (Greaves and Khalifa, 2004). Pruritus is a distressing sensation or even a psychological disorder that substantially impair the quality of life (Finlay and Burgdorf, 2004). AD patients have a lower threshold of itch than normal non-infected skin and are inherently itchy (Morren *et al.*, 1994). Most often, the sensation of pruritus impairs the quality of life in numerous manners and extent (Daud *et al.*, 1993;Jemec and Wulf, 1996). Pruritus can be evoked or augmented by a vast variety of physical, chemical and pharmacological stimuli (Table 1.2). Among all, peripheral pharmacological mediators plays a key role in the production of itch in atopic dermatitis (Greaves, 2004). It has also been reported that chronic psycho-emotional stress and worry would involve cortical centers and activate the hypothalamus-pituitary-adrenal axis, thus enhancing sensation of pruritus (Slominski and Wortsman, 2000;Paus *et al.*, 2006b).

1.4.2 Difference between pruritus and pain

There always exist controversial arguments on the actual pathophysiology of pruritus, which, as a result, hinder researchers on tackling this important frustrating symptom of atopic dermatitis (Yosipovitch *et al.*, 2003). While itch and pain are transmitted in different specific peripheral C-units and central afferent pathways (Baron

et al., 2001), there exist complex interactions and similarities between pruritus and pain (McMahon and Koltzenburg, 1992;Schmelz, 2005), only differ at the outcome: pain brings out a reflex withdrawal, whereas pruritus leads to a chronic reflex of scratching. Another group of scientists distinguish itch and pain as 2 completely different sensations by using electrical stimulation via a microelectrode implanted in afferent nerves of patients (Hsieh *et al.*, 1994). Both pain and itch can be reduced by soft rubbing, which activates fast-conducting, low-threshold nerve fibers (Bromm *et al.*, 2000).

1.4.3 Pathogenesis and neuronal pathways of pruritus

That pain and itch are transmitted along the same nerve pathways proposed before has been prevailing until recently, when dedicated itch-transmitting neurons were discovered at both the peripheral and central afferent pathways (Schmelz *et al.*, 1997;Andrew and Craig, 2001b) (Figure 1.3). The most widely accepted theory of neuronal pathophysiology of pruritus nowadays is an independent, yet almost identical neural pathway of pruritus and pain sensations (Yosipovitch *et al.*, 2003). Inflammatory skin lesions of AD patients would trigger the dermal mast cells in order to form or release a bunch of mediators, including neuropeptides, neurotransmitters, proteases, arachidonic acid derivates, cytokines and skin-infiltrating T-cells, etc., at cutaneous region (Stander and Steinhoff, 2002) (Figure 1.4). A more detailed collection of physical, chemical and pharmacological stimulations is listed in table 1.2. Afferent itch-dedicated polymodal C neurons were discovered upon induction of itch by histamines (Schmelz, 2001), which are distinct form pain-transmitting fibres. A signal is generated upon binding of various mediators to pruritoceptive endings of these C fibres and a signal is generated. The signal

is being transmitted at a slow conduction velocity via specific secondary transmission neurons in the lateral spinothalamic tract of the dorsal horn, projecting to the thalamus for interpretation of sensation (Andrew and Craig, 2001a). Pruritus signals were then found to co-activate the anterior cingulated cortex, inferior parietal lobe, supplementary motor area and also several forebrain regions in the brain (Darsow *et al.*, 2000;Drzezga *et al.*, 2001;2001).

The mechanisms of pruritus mentioned above were mainly focused on inflammatory sites. Yet there also exists itching related to chronic lichenification from excessive drying of the skin in AD patients without noticeable lesions. Emollients are all that are required for management of xerosis and will be inadequate where inflammatory changes are responsible for the itching. A clear difference between these 2 sorts of pruritus has to be distinguished.

1.4.4 Neurogenic itch

However the story is not so straightforward. It has been found out that non-lesional eczematous skin evokes a significantly lower degree of pruritus than lesional regions. The complex combination of a inhibitory degree of afferent neuronal sensation in non-lesional skin and elevated itch response in lesional region will lead to a defective modulation of itch traffic, thus enhancing itch process in the CNS, leading to central sensitization and amplified itching perception, hence exemplifying itching and generating an important category of neurogenic itch (Twycross *et al.*, 2003;Yosipovitch *et al.*, 2003;Greaves and Khalifa, 2004). It has also been proved that in cases of chronic skin inflammation, activation of nociceptors not only provokes itch, buy also facilitates itch

processing in the spine, resulting in touch-evoked pruritus around an itching site (Paus *et al.*, 2006a). In such cases, misinterpretation of even normally painful stimuli as itch will occur (Ikoma *et al.*, 2003). The idea of neurogenic itch explains the causation of persistent itching sensation by changes in non-itchy mechanical stimuli (alloknesis), such as sweating and temperature change (Heyer *et al.*, 1995).

1.4.5 Role of histamines in pruritus

Among all mediators, histamines have long been believed as the major peripheral mediator in pruritus since the very beginning of skin research (Lewis, 1927). Traditional approach of pruritus treatment relied heavily on antihistamines, however scientists also argued its usage based on the result of little or no effect of histamine receptor antagonists in relieving itch, especially in AD (Rukwied *et al.*, 2000). It has also been argued that the effectiveness of antihistamines are due to their sedative actions or other poorly known pharmacological properties rather than peripheral histamine-blocking activity (Behrendt and Ring, 1990;Munday *et al.*, 2002;Rees and Murray, 2005).

1.4.6 Substance-P

Due to the non-specific nature of histamines, such measurements are not satisfactory in determining the extent of pruritus in AD patients. Therefore scientists continue to search for other key mediators. One of the classes of mediators is tachykinins, which are located in unmyelinated sensory nerve fibres in skin. Among them, substance P (SP), which is a 11-amino-acid neuropeptide, causes redness, whealing and itching (Hagermark *et al.*, 1978;Jorizzo *et al.*, 1983) and is important for direct communications

between mast cells and nerve fibres (Suzuki *et al.*, 1999). In AD patients mast cells play a pivotal role in triggering acute hyperresponsiveness once received signal from Th-2 cells. The vast amount of tryptase secreted by activated mast cells during acute inflammation will cleave proteinase-activated receptors 2 (PAR2), which are richly expressed around afferent nerve terminals. SP will be produced at the dorsal root ganglion of nociceptor C fibres upon such a cleavage, hence transmitting and amplifying pruritic signals (Steinhoff *et al.*, 2000). High concentrations of SP will also bind back to the specific NK1 receptors of the mast cell, causing their degranulation and subsequent release of pruritic mediators (Yosipovitch *et al.*, 2003), including tumor necrosis factor –α (TNF-α), which will further stimulate the endings of nociceptors (Cocchiara *et al.*, 1999). Thus a positive feedback loop is generated, amplifying signals to a greater extent.

1.4.7 Brain-derived neurotrophic factor and other recent mediators in pruritus

Another important pruritogenic mediator that is under heavy investigation is brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family (Leibrock *et al.*, 1989) richly found in the neurons (Lewin and Barde, 1996). Neurotrophins were found to have mediatory effects upon inflammatory conditions (Toyoda *et al.*, 2002), and were also suspected in regulating important immune cells of AD, including T-cells (Kerschensteiner *et al.*, 1999) and eosinophils (Raap *et al.*, 2005). Its multifactorial effect may therefore shed a new light on the understandings of pruritus.

1.5 Scratching, nocturnal scratching and sleep behavior in atopic dermatitis subjects and related research progress

1.5.1 Overview

Pruritus and sleep problems are directly interrelated and AD patients with severe itching are prone to difficulties falling and staying asleep over a whole night than those without such problems (Stores *et al.*, 1998;Finlay and Burgdorf, 2004). The primary reason for such a relationship between pruritus and sleep loss is due to the change in temperature based on the concept of alloknesis (Heyer *et al.*, 1995), with a warmer environment in bed being a mechanical stimulation to pruritus-specific C neurons. In paediatric patients, their sleep abnormalities is a combination of initialization of sleep and failure in maintaining a tight sleep throughout, leading to poor sleeping conditions, with consequent negative impact on their schoolwork, impairments on their temper and their family relationships during the daytime (Reid and Lewis-Jones, 1995;Klein and Clark, 1999). Parents may also suffer from sleep deprivation and exhaustion (Reid and Lewis-Jones, 1995). As a result, measurements of both parameters serve as an important role in evaluating disease severity and QoL impairment of AD patients (Finlay and Burgdorf, 2004).

The extent of sleep loss was assessed traditionally by visual analogue scales or questionnaires, both of which relied heavily on whether both subjects and their caregivers were able to relate their perceived experience accurately (Hon *et al.*, 2003). However, self-reports are always inaccurate because nocturnal sleep disorder is not a conscious activity. Even when conscious, the required information was always poorly remembered when combining the therapeutic effects of sedative drugs (Benjamin *et al.*, 2004).

Bypassing the ambiguity, scientists use the extent of scratching as another important quantification parameter to define itch, basing on the fact that scratching is the main response being provoked by the itching sensation.

1.5.2 Interrelationship between pruritus, scratching and sleep disturbance

In order to use scratch measurement to replace itch measurement, their interrelationship have to be clarified. Scratching is a voluntary motoric action to counteract the pruritic sensation by application of slightly painful stimuli (Paus *et al.*, 2006a). It is the primary action for alleviating itch in patients (Greaves and Khalifa, 2004). The action of scratching or rubbing will stimulate a group of touch and pressure-conducting myelinated A neurons via low threshold mechanoreceptors. Such stimulation is antagonistic to that of pruritus, thus surrounding itch processing by inhibitory neuronal circuits in substantia gelatinosa in the grey matter of the spinal cord is leveled off (Kirchner *et al.*, 2002). Hence the modulatory signal towards dorsal horn is reduced, and so does the perception of itch (Wall and Melzack, 1995;Greaves and Wall, 1996). Last but not least, vigorous scratching would generate new wounds, thus triggering a cascade of inflammatory mediators, leading to a new wave of pruritus sensation through the mentioned neurogenic pathways. The itchier the patients get, the more they scratch consequently, and thus a vicious cycle is generated. Therefore the relationship of itch and scratching are inseparable, and measurement of scratch would be a sounding approach for replacing subjective pruritus measurement, basing on the argument that the higher the intensity of pruritus, the more the patients will scratch in order to alleviate the sensation.

Among all aspects of life quality that are impaired by atopic dermatitis, sleep disturbance by pruritus and nocturnal scratching is the most important area of research (Stores *et al.*, 1998;Reuveni *et al.*, 1999;Chamlin *et al.*, 2005). Pruritus is always associated with sleep disturbances in children (Dahl *et al.*, 1995;Stores *et al.*, 1998). Moreover, difficulty falling asleep and night waking in children with AD correlate with daytime behavioral problems (Dahl *et al.*, 1995). As a result, sleeping behavior of AD patients should never be neglected.

1.5.3 Current methodologies in nocturnal scratching and sleep quality measurement

Despite its significance in exacerbating AD situation, scratching due to pruritus is difficult to be documented in the home environment of patients (Rees and Laidlaw, 1999;Hon *et al.*, 2006a). Traditional assessments of sleep and nocturnal pruritus in children with AD include infrared videotaping (Ebata *et al.*, 1996) and overnight polysomnography (PSG) (ncoli-Israel *et al.*, 2003), which has been applied on AD patients and a recording of overnight sleep physiology, is marked by frequent awakenings associated with scratching episodes and an overall reduced sleep efficiency is observed (Stores *et al.*, 1998). However, the practices are rather difficult to be carried out in a home-base environment for routine, continuous and repetitive measures (Ebata *et al.*, 2001). The patients often find them uncomfortable (Hon *et al.*, 2006a). Another measurement approach focused on movement detection devices and meters that can record and quantify scratch, the objective correlate of itch. Such a research direction began in 1970s and is still ongoing today (Savin *et al.*, 1973;Felix and Shuster, 1975;Bender *et al.*, 2003;Benjamin *et al.*, 2004;Hon *et al.*, 2006a). Such devices have

become more computer-friendly since the establishment of actigraphy techniques (Cole *et al.*, 1992), which is being generally defined as a method for measurement of activity through small, wristwatch-like devices (Acebo and LeBourgeois, 2006). Actigraphy allows easy transfer of data and signals from the device into computer for analysis. Scratching movement can also be expressed in a visual pattern with contours. Such accelerometers have been validated against the gold standard of infrared videotaping of children with AD in their own homes (Benjamin *et al.*, 2004). Adequate agreement between actigraphic data and sleep scored from PSG recordings were also met (Sadeh and Acebo, 2002). Yet there still leaves a drawback that it is very difficult to distinguish between scratching activity and limb restlessness from the whole bunch of data obtained (Bringhurst *et al.*, 2004). It is difficult to discard those data that are not related to sleep, i.e. artifacts recorded by actigraphy (Acebo and LeBourgeois, 2006). Even for recording at night only, the dissociation among the entirely different sorts of nocturnal activities is not possible. To date, there have been no standardized protocols for sleep recording, data acquisition and other related variables. New users often find it difficult to obtain a published validity study for these new devices (Acebo and LeBourgeois, 2006).

Major criteria

- Pruritus
- Typical morphology and distribution:
  Flexural lichenification or linearity in adults. Facial and extensor involvement in infants and children
- Chronic or chronically relapsing dermatitis
- Personal or family history of atopy
  (asthma, allergic rhinitis, atopic dermatitis)

Minor criteria

Xerosis
Ichthyosis / palmar hyperlinearity / keratosis pilaris
Immediate (Type I) skin test reactivity
Elevated serum IgE
Early age of onset
Tendency towards cutaneous infections
Tendency towards nonspecific hand or foot dermatitis
Nipple eczema
Cheilitis
Recurrent conjunctivitis
Dennie-Morgan infraorbital fold
Keratoconus
Anterior subcapsular cataracts
Orbital darkening
Facial pallor / facial erythema
Pityriasis alba
Anterior neck folds
Itch when sweating
Intolerance to wool and lipid solvents
Perifollicular accentuation
Food intolerance
Course influenced by environmental / emotional factors
White, dermographism / delayed blanch

Table 1.1 Diagnostic criteria for atopic dermatitis in children and adults. A patient should have at least three major criteria accompanied by at least three minor ones. (Hanifin and Rajka, 1980)

Physical /Mechanical
Light touch
Stroking
Vibration
Mild heat
Electrical

Chemical
Acids
Alkali
Other irritants

Pharmacological
Histamine
Histamine liberators
    -Morphine
    -Codeine
    -Compound 48/80
Serotonin (5-hydroxytryptamine)
Prostaglandins
Platelet-activating factor
Kallikrein
Cytokines
    -Interleukin-2
Proteases
    -Trypsin
    -Papain
    -Mucunain
Tachykinins
    -Substance P
Calcitonin gene-related peptide
Opioid peptides
    -β-endorphin
    -Leu-enkephalin
    -Met-enkephalin

Table 1.2 Main Classes (with examples) of externally applied factors which cause itching (Greaves, 2004).

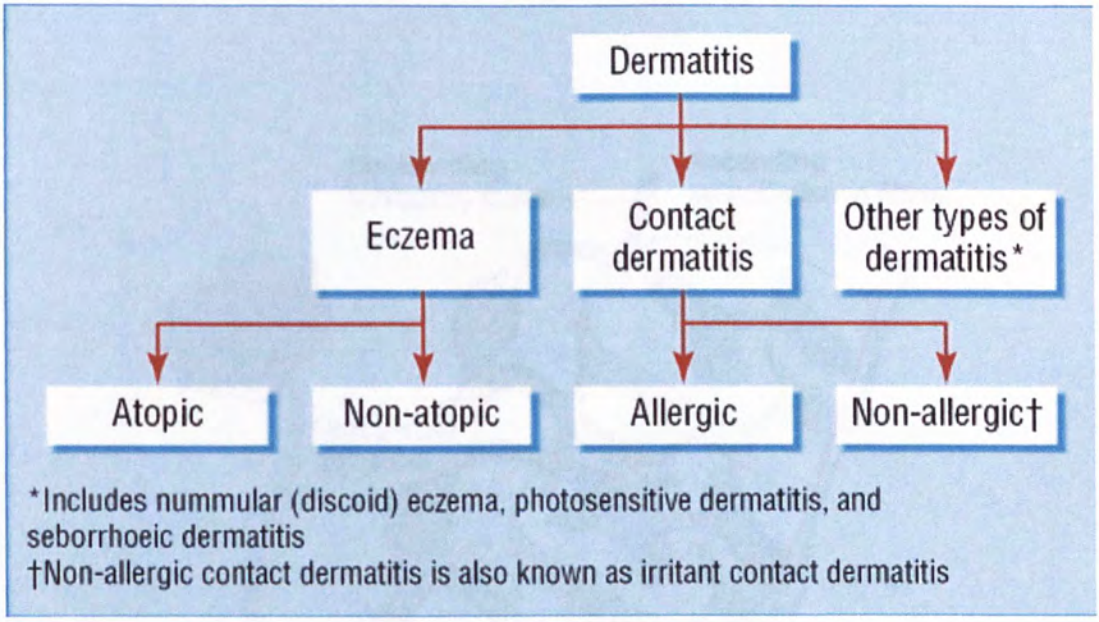


Figure 1.1 Subgroups of dermatitis. Some patients may have a combination of subgroup types. (Brown and Reynolds, 2006)

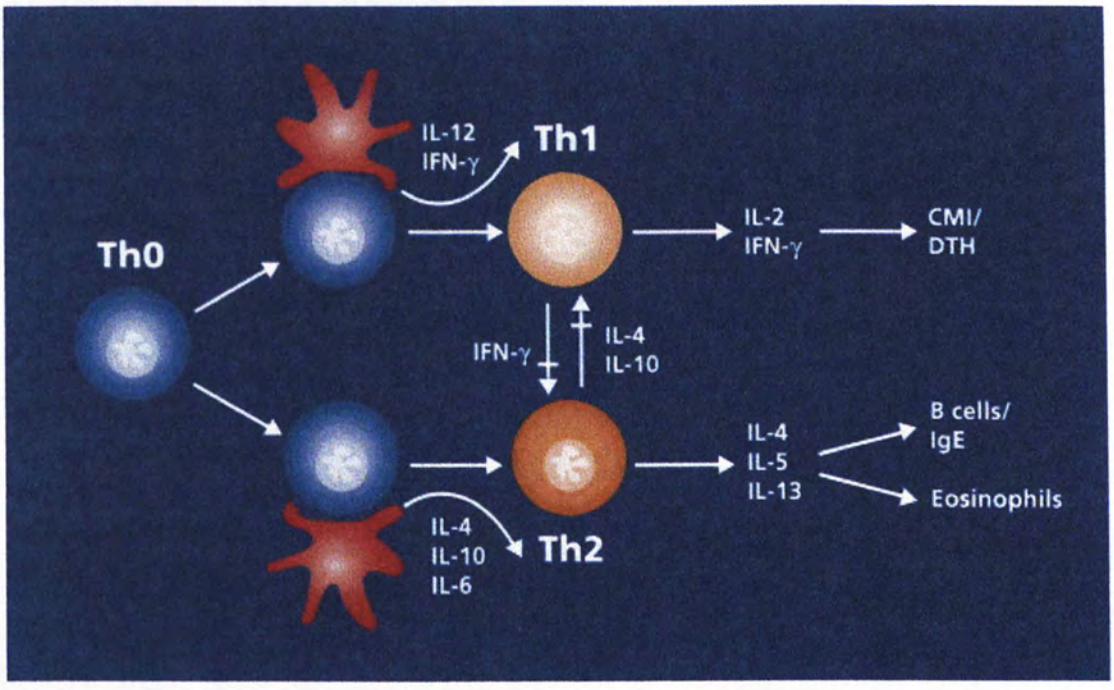


Figure 1.2 Differentiation of T-helper *(Th)* lymphocytes (Friedmann, 2002).

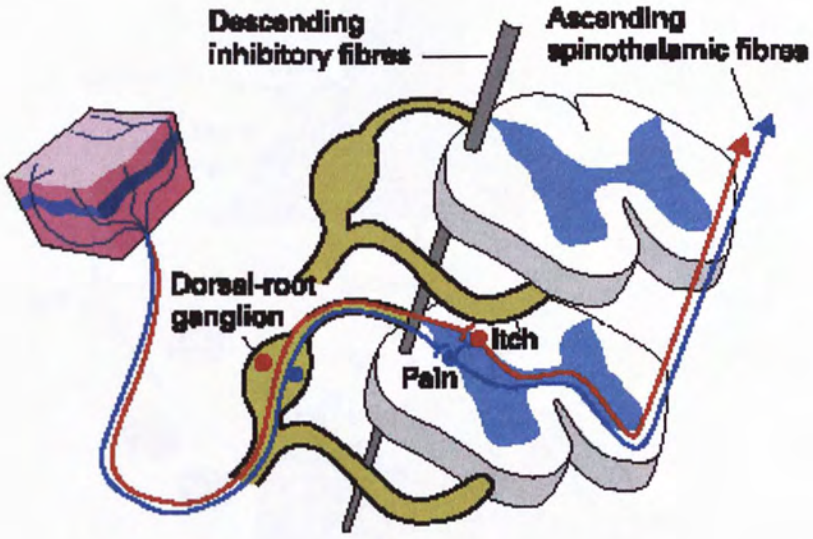


Figure 1.3 Neurophysiology of itch (Yosipovitch *et al.*, 2003). Signals are generated first at the nerve endings in cutaneous region, then pass through the unmyelinated slow-conducting C fibres, dorsal root ganglion and the transmission neurone.

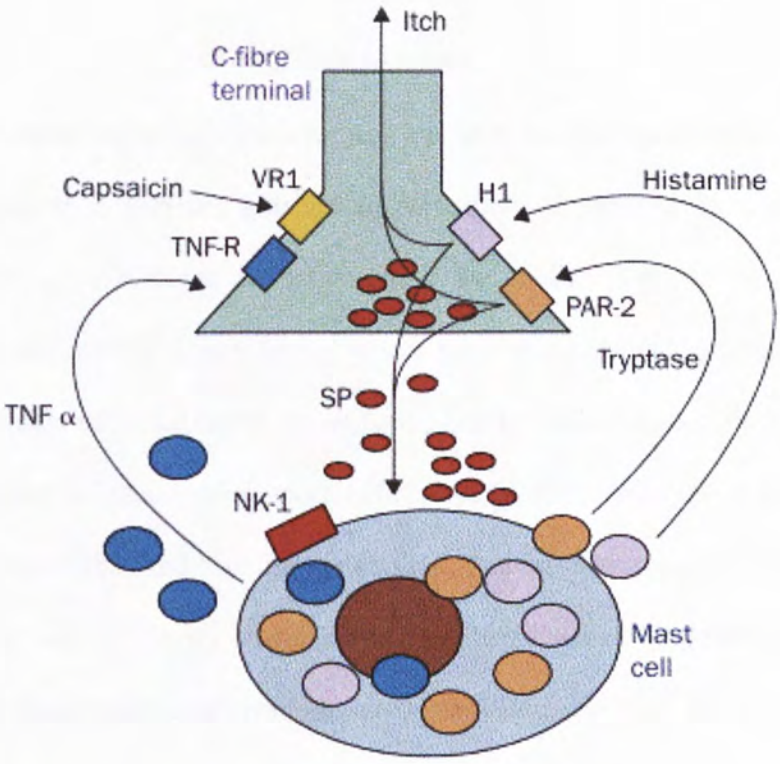


Figure 1.4 Cross-talk between cutaneous afferent C neuron terminals and dermal mast cells (Yosipovitch *et al.*, 2003). A collection of mediators are released from dermal mast cells, which bind to pruritoceptive endings of C fibre endings, thus generating signals of pruritus.

Chapter 2

Objectives

The primary objective of this project was to investigate the possibility of using an alternative method to measure pruritus, in particular nocturnal scratching in paediatric patients suffering from atopic dermatitis (AD). The methodology should be easy to be carried out at the subject's own home, where the routine sleeping conditions could be reflected. The data obtained had to be objective and be able to truly reflect their sleeping conditions in home-based environment rather than in completely unfamiliar conditions. Objectivity was emphasized because the traditional questionnaires or reports, in the form of a sleep diary, relied heavily on subjective reporting from either patients or caregivers. Nevertheless, these traditional methods would still be performed for comparisons with the new measurement. Examinations were done by one investigator only throughout to avoid intra-observer variability. In addition, diagnosis of AD for all patients would only follow one criterion throughout the whole study, i.e. the criterion proposed by Hanifin and Rajka (Hanifin and Rajka, 1980) (Table 1.1) and by performing measurement of AD severity using one scoring system, the widely used SCORing Atopic Dermatitis (SCORAD) index (Kunz *et al.*, 1997).

The device used in the new methodology should be non-intrusive to the patients in the long run. As for validation purpose, blood was taken throughout the whole study to obtain a series of objective laboratory markers to look for any close relationship with the data obtained. To achieve this, written consents were taken from the participants and the whole protocol was approved by the Clinical Research Ethics Committee of the university. Various relationships among the data obtained from the new methodology

with the SCORAD index and all objective clinical markers were investigated by statistical means to look for significant inter-relationships.

The secondary objective was to test the device in clinical trials through investigating the efficacies of various AD medications, especially on the alleviation of sleep problems and improvements in quality of life. In particular, medications other than traditional therapies of topical corticosteroids, such as traditional Chinese herbal medications and calcineurin inhibitors (e.g. tacrolimus ointment), would be used and their effectiveness would be discussed in depth below. Pruritus involved in diseases other than AD, if any, was also one of the areas of interest of this study.

By investigating all of the parameters mentioned above, we aimed at defining a new paradigm for itch measurement, clinical manifestations of itch and its impairment with quality of life on AD patients, and the application of our findings in certain medication trials. All these were done to achieve the ultimate aim: to take a step in relieving the chronic suffering of children irritated by itch.

Chapter 3

Methodologies and Materials

3.1 Validation of a new methodology / device

3.1.1. Device selection

Based on the criteria set for a new device mentioned above, various measuring devices were considered including accelerometers, infrared videotaping and overnight polysomnography. Digitrac® Limb Movement Recorder (IM Systems, Baltimore, MD) (Figure 3.1) was chosen at the end. It is a light-weight, self-contained, wrist-worn device that can be easily worn on the limb even for young patients. This is particularly useful in our study for young patients to assess their nocturnal scratching behavior in a home-based environment. It is completely ambulatory and no wire connection is necessary during recording of motion. With the help of a piezoceramic acceleration sensor, the device can record not only the amplitude, but also frequencies of limb movements in a 3-dimentional manner. The Digitrac Limb Movement Recorder has been used in sports science research and this was the first time for it to be applied in the field of medical science.

3.1.2 Study design

Patients aging 2-18y, with AD diagnosed according to the Hanifin (Hanifin and Rajka, 1980) criteria, were recruited from the paediatric dermatology outpatient clinic of our university-affiliated teaching hospital. Severity and symptomatology (pruritus and sleep loss) of AD in the preceding three days were evaluated with the aid of the SCORAD index (Kunz *et al.*, 1997) (Figure 3.3). The components in the SCORAD

scores provided information on the extent, types and severity of eczematous lesions. Examinations were performed by only one investigator to avoid any intra-observer variability. Subjects with a SCORAD score ≥ 15 were enrolled into the study. Subjects were excluded if the patients' caregivers failed to provide information regarding pruritus or sleep loss, or if obvious parasomnias were evident from history. Any sedative sleeping medications were also prohibited one week prior to the study and during the night of motion recording. Normal children seen at the clinic with non-inflammatory and non-itchy skin conditions as well as healthy children from hospital staff were recruited as controls. All subjects were then instructed to wear the device on their dominant wrist before going to bed.

In the pilot testing, Digitrac was found to be able to obtain data on a wide spectrum of frequencies of wrist movements and quantify movements in term of acceleration or *g* values. The monitor was programmed to record limb motion between 10 p.m. and 8 a.m. in the next morning, only the data from the time- the patients had got in were analysed. In order to avoid any artifacts being analyzed, patients were also required to give a verbal report on the time getting asleep and waking up. All data were stored automatically into a flash memory within the recorder, and the device was collected the next day with the data being downloaded to computer using the program DigiTrac 3.9 (IM Systems, Baltimore, MD) (Figure 3.2). Wrist activities were expressed in unit of average value of acceleration (g min^{-1}). With the aid of the program, 3-dimensional frequency-specific contourgraphs of limb motions were obtained, and the data was expressed in mg min^{-1}.

The average limb activities frequency contour spectra of AD patients and controls were assessed to determine any significantly different patterns between the two parties by performing student t-tests. A Pearson's correlation test among the subjective and objective component of the SCORAD scores and Digitrac data of the patients were also performed for any significant relationship. In particular, focus will be put on comparing the objective data recorded by the device and the subjective symptom scores.

3.1.3 Validation of the Digitrac with laboratory markers

As for the validation of Digitrac, laboratory markers were also used for cross-reference. The necessity of blood-taking has also been included in the written consent mentioned previously. Venous peripheral blood (PB) was collected from recruited AD patients, stored in serum separator and EDTA blood collection tubes (Greiner Bio-One, Kremsmuenster, Austria). Serum concentration of total IgE was measured by micro-particle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL), and results were logarithmic transformed before analysis. Eosinophil counts in PB were enumerated using Coulter STKS counter (Beckman-Coulter, Miami, FL). Both assays were done immediately after blood collection.

The rest of blood collected was centrifuged at 4°C and 2041×g for 10 minutes within 2 hours after collection using a Himac CR7 centrifuge (Hitachi Koki, Ibaraki, Japan). Serum (from serum separator tubes) and plasma (from EDTA tubes) obtained as the supernatant after centrifugations of the blood samples were stored at -80°C until analysis of markers in one batch.

Thymus and activation-regulated chemokine (TARC) and cutaneous T-cell

attracting chemokine (CTACK), being chemotactic factors for AD-associated leukocytes, are used as objective laboratory parameters to validate Digitrac in this study. Plasma concentrations of TARC and CTACK were measured in duplicates by two enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN) (Figure 3.4a). The lower limits of detection for TARC, CTACK were 7 and 1.5 pg ml^{-1}, respectively.

The two ELISA protocols were similar. During the actual assay, 100 μl of assay diluent was added to each well of a microparticle plate pre-coated with monoclonal antibody specific for the markers, followed by the addition of 50 μl standards or samples into each well. The plate was incubated at room temperature for 2 hours, by the time any TARC / CTACK present in either samples or standards were bound by the immobilized antibody on the plate. After aspiration by washing buffer to remove unbound substances, 200 μl of enzyme-linked monoclonal antibody specific for the markers to be studied was added into the wells. The whole plate was again incubated for 2 hours to allow for the formation of sandwich conjugates between the enzymes and chemokines present. Following a wash to remove any unbound antibody-enzyme reagents, 200 μl of a solution of hydrogen peroxide and tetramethylbenzidine was added to the wells and color was allowed to develop within 30 minutes in proportion to the amount of TARC / CTACK bound in previous steps. The color development was stopped by addition of 50 μl stop solution (2N sulfuric acid) and the intensity of the color of each well is measured with a microplate reader with a μQuant plate reader (Bio-Tek Instruments, Inc., Winooski, Vt.) (Figure 3.4b), under a wavelength of 450 nm, with wavelength λ correction at 540 nm. A standard curve of TARC / CTACK concentrations was obtained and the actual concentrations of each well were then calculated basing on the standard curve plotted and

dilutions of samples done for the assay.

3.1.4 Factor and statistical analysis

In order to explore the correlations among different subjective and objective parameters and to confirm they are independent dimensions in assessing AD severity, exploratory factor analysis was performed using the Statistical Package of Social Sciences (SPSS) (v.13.0, SPSS Inc., Chicago, IL, USA). Bartlett's test of sphericity was used to assess the possibility for performing factor analysis, and Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was also evaluated. A high KMO (maximum 1.0; minimum acceptable 0.5) indicates that data is likely to factor well since correlations between pairs of variables can be explained by the other variables (low partial correlation coefficients). Correlation coefficients were analyzed by principal component analysis and subsequent rotation according to the standard varimax criterion (Stevens, 1996;2002). It was decided *a priori* that the number of factors in the varimax rotation would be based on the number of eigenvalues ≥ 1.0 in the principal component analysis (Cattell, 1966).

All data obtained was analyzed with the program SPSS 13.0. Data were expressed as means with standard deviations (SDs) or standard errors of mean (SEMs). Parametric statistical tests were performed if the data showed normal distribution, Pearson correlations were performed to look for any significant relationship between two variables. A two-tailed p-value of less than 0.05 was considered to be statistically significant.

3.2 Application of Digitrac in traditional Chinese herbal medication (TCHM / TCM) clinical trial

3.2.1 Current AD treatment using corticosteroids and their drawbacks

The mainstay of AD treatment has remained unchanged for several decades, and still relies heavily on corticosteroids (CSs) nowadays (Judge, 2005) in both systemic and topical formulations. However, CS treatment is not specific enough. Prolonged usage of CSs have a wide range of suspected immunomodulatory / immunosuppressive effects including suppression of cytokine synthesis, leukocyte chemotaxis and adhesion molecule expression (Pucci *et al.*, 2000). CS is also linked with potential threats of deranged metabolism, growth suppression and increased susceptibility to infection. Potent topical CS application can result in significant suppression of the hypothalamic-pituitary-adrenal axis, altered linear bone growth, or even growth retardation in children (Ellison *et al.*, 2000;Schachner *et al.*, 2005). These adverse effects together with the emotional fear of long-term use of topical steroids have induced topical steroid phobia in many patients throughout the world (Charman *et al.*, 2000). This often drives patients to seek for alternative therapies, and traditional Chinese herbal medications (TCM) is one of the most commonly used treatments (Hon *et al.*, 2004b).

3.2.2 Recent trend on TCM treatment

Traditional Chinese Medicine focuses on the importance of catering the therapy to suit the different needs of each individual, whereas most Western medication trials are standardized and stress on "average" efficacy in large, double-blind, placebo-controlled studies. This difference hinders TCM research in both inpatient and outpatient settings

(Koo and Desai, 2003). Although TCM is widely used in many Asian societies, its beneficial effects on children with AD have not been consistently demonstrated and undesirable side effects have been reported (Hon *et al.*, 2004b).

Currently there are only a few well-documented studies on the application of Chinese herbal therapy in AD (Sheehan *et al.*, 1992), while none of the available works was well-validated with combinations of objective measures, especially for pruritus. Therefore, the next step in this study aimed at evaluating the clinical effects of TCM on AD severity, pruritus and life quality of the patients by objective measures, including the application of the Digitrac movement recorder. Possible adverse events of our TCM on haematologic, hepatic and renal functions, if any, were also recorded to ensure safety of the medications.

3.2.3 Study plan

The TCM formula used in the trial was named as Pentaherbs (Figure 3.5). It was synthesized in the form of capsules for the convenience of the patients and to eliminate the taste of bitterness. The original formula was: *Flos lonicerae* (Jinyinhua) 2 g, *Herba menthae* (Bohe) 1 g, *Cortex moutan* (Danpi) 2 g, *Rhizoma atractylodis* (Cangzhu) 2 g and *Cortex phellodendri* (Huangbai) 2 g (total 9 g of raw herbs daily). The extraction rate from this raw herb was around 18%–20%, and the extracted portion was made into six to seven capsules. The dose calculation was based on the assumption that patients had to be old enough to swallow the capsules without difficulty (between 7 to 12 years of age). The capsule was manufactured, packaged and labeled by the Chinese Medicine Industry Development Centre of the Hong Kong Institute of Vocation Education, using Good

Manufacturing Practice (GMP) standard. The optimal composition of each herb included was standardized prior to manufacturing. The powder was formulated into uniform dose capsules under the supervision of the Clinical Trials Section, Institute of Chinese Medicine of The Chinese University of Hong Kong according to established procedures. Before the clinical trial, these capsules were assessed by the Institute of Chinese Medicine for the presence of heavy metals, microbial products and residual pesticides to ensure that the quality and safety requirements were met.

Prior to the study, patients were required to complete the Children's Dermatology Life Quality Index (CDLQI) in the form of a questionnaire in order to assess the subjects' life quality impairment (Lewis-Jones and Finlay, 1995) (Figure 3.6). The CDLQI score consists of 10 questions, with each question scoring from 0 ("not at all") to 3 ("very much"). The maximum possible score of the whole questionnaire is 30 and the minimum score is 0. The higher the score is, the more the quality of life (QoL) is impaired. To facilitate a better understanding of the questionnaire to the subjects, a Cantonese-translated version was used (Chuh, 2003) (Figure 3.7).

Participants underwent a 4-week run-in period. During this period, their baseline dietary intake, emollient and drug use, and information on severity of AD were collected. AD of the subjects was diagnosed according to the Hanifin criteria (Hanifin and Rajka, 1980), and would be excluded if they had active concurrent disease other than allergic diseases such as asthma and allergic rhinitis, or if they had received psoralen ultraviolet A (UVA) treatment within the previous 8 weeks. The SCORAD index was performed by the investigator at baseline (1993). A SCORAD score of less than 15 was considered to be too mild to be enrolled into the study. The patients would then be given three Pentaherbs

capsules (250 mg active herbs each) twice daily for a consecutive 12-week period. Physicians at the Institute of Chinese Medicine of our university considered this dosage as suitable for a wide range of age groups. Correct use of the medications was explained to each subject and their parents, thus ensuring that they were compliant to the treatment regimen and proper use of medications. Enrolled subjects were followed up once every 6 weeks. Each patient was given an Eczema Diary at the beginning of the trial for recording daily symptoms and adverse effects, if any. The severity of AD was assessed with the SCORAD index during each visit, and this served as the primary outcome of the study. Patients were allowed to continue routine relieving medications like CS and antihistamines throughout the study yet these concurrent treatments had to be kept unchanged throughout the study. Adherence to the study regimen was evaluated by comparing the number of capsules returned with the number recorded as taken in the Eczema Diary at the end of the trial (Appendix I).

Subjects were instructed to wear the device on the dominant wrist before getting to bed on the first night of the baseline of the trial. The setting, collection and analysis of data from the device followed the ones used in the validation stage. The same procedure was being carried out during the end of the study.

3.2.4 Validation with laboratory markers, Staphylococcus aureus infection and statistical analysis

As for the clinical markers, PB was obtained during the clinic visit at baseline and the completion of the study. IgE levels and eosinophil counts were measured using the same protocol mentioned above. Plasma concentrations of CTACK and TARC were

obtained using the same ELISA protocol (R&D Systems, Minneapolis, MN). In addition, concentrations of pruritic markers, brain-derived neurotrophic factor (BDNF) and substance P (SP), were also investigated before and after treatment. This was achieved by another 2 ELISA assays (R&D Systems, Minneapolis, MN; Sigma-Aldrich, Saint Louis, Missouri).

The study also took colonization of *Staphylococcus aureus* (*S. aureus*) into consideration in order to look for any correlation between the degrees of bacterial colonization and AD severity. Swabs (COPAN innovation, Italy) were taken from the patients' anterior nares, flexures (anterior neck, antecubital fossae and popliteal fossae) and the worst inflammatory skin areas (defined as lesional skin with oozing or crusting), with 5 seconds of rubbing in each region. Bacterial cultures of the swab samples were carried out by standard laboratory techniques and the sensitivity of any bacterium towards commonly used antibiotics were examined (Department of Microbiology, the Chinese University of Hong Kong). Bacterial growth was classified as scanty ($<10^4$ colony-forming units per mL), moderate (10^4-10^5), or heavy ($>10^5$). We defined moderate-to heavy growth as significant growth, and scanty or nil growth as negative.

The results on SCORAD and laboratory parameters in paired samples before and after Pentaherbs use were compared using the Paired-samples t tests by SPSS 13.0. The correlations between SCORAD and inflammatory markers were analyzed by Pearson correlation coefficients. All comparisons were two-tailed, and p values less than 0.05 were considered significant.

3.3 Application of Digitrac in a trial of 0.1% tacrolimus ointment in treatment of atopic dermatitis

3.3.1 Topical immunomodulators as a treatment approach of AD

Another new therapeutic alternative of AD under rapid development over the past decade is the non-steroid immunosuppressive drugs, or topical immunomodulators (TIMs). They were originally being used as transplantation medicine. FK506 was the first one being used in treating dermatological conditions. It is a 822 kDa macrolide lactone, which is unstable in aqueous solution and has another name in this form: tacrolimus (Nghiem *et al.*, 2002) (Figure 3.8). It is a short polypeptide produced by *Streptomyces tskubaensis.* Tacrolimus has its greatest penetration power upon inflamed skin sites (Ruzicka *et al.*, 1997), and is shown to have a three times greater affinity towards cytoplasmic immunophilin FK-binding protein (FKBP) than another common TIM, pimecrolimus (Reitamo *et al.*, 2002).

3.3.2 Mechanism of tacrolimus in suppressing AD and pruritus

In the immunological pathway of T-cells, calcineurin is a calcium-activated phosphatase enzyme responsible for dephosphorylation of the nuclear factor for activated T-cells (NF-AT). NF-AT is important in promoting transcription and differentiation of T-cells into T_H1 or T_H2 types (Novak *et al.*, 2005). Tacrolimus exhibits an inhibitory mechanism on the enzyme calcineurin by binding it with a complex of tacrolimus-FKBP12, calcium and calmodulin (Baldo *et al.*, 2005). After this binding process, the transcriptions of NF-AT-dependent genes are turned off, resulting in down-regulation of T-cell activities. Therefore, TIMs are also commonly known as calcineurin inhibitors.

Tacrolimus has also been found useful in the blockade of interleukin-2 transcription (Dumont, 2000) and down-regulation of T-cell Fas ligand expression (Shaw *et al.*, 1995), thus suppressing immunological responses in patients with AD.

On the aspect of pruritus, tacrolimus might pose an anti-pruritic effect by its anti-inflammatory effect because lesional AD skin often shows a dense inflammatory cell infiltrates together with an accumulation of neuropeptides that are known to mediate or aggravate pruritus (Stander and Luger, 2003;Stander *et al.*, 2003). This is why the side effects of pruritus, burning and skin sensations are common in the initial phase of TIM treatment (Novak *et al.*, 2005). TIMs are also useful in blocking signal transduction in targeting neuronal cells including interleukin-2, which is a known mediator of pruritus, thus reducing the sensation of pruritus (Hanifin *et al.*, 2001;Rigopoulos *et al.*, 2004;Stander and Steinhoff, 2002). However, several tacrolimus clinical trials showed that mild pruritus is one of the adverse effects of the medication (Bekersky *et al.*, 2001;Paller *et al.*, 2001), yet the occurrence of this symptom would decrease after the first few days of the treatment. Based on such a controversy, the Digitrac monitor was applied at this stage to objectively measure pruritus and the scratching behavior in AD patients upon application of tacrolimus, thus evaluating the feasibility of using such medication in relieving itch.

3.3.3 Study plan

The study was a case series and no controls were recruited. Emphasis is put on the use of Digitrac technique to detect the efficacy of tacrolimus ointment in alleviating pruritus and sleep loss in AD patients. The study was carried out in the Department of

Paediatrics of The Chinese University of Hong Kong. Written consents were obtained from parents for both the use of 0.1% tacrolimus ointment (Fujisawa Healthcare, NY) (Figure 3.9) and Digitrac during the treatment. AD patients, aged 2 to 18 years, from the outpatient clinic of the Department were recruited for the pilot study. AD was diagnosed according to criteria proposed by Hanifin and Rajka (Hanifin and Rajka, 1980). The clinical severity of AD was evaluated according to the SCORAD score. All subjects had an objective SCORAD $\geq$ 15 at the entry into this study. Among them, those who had active concurrent disease (except allergic diseases such as asthma and allergic rhinitis), received psoralen UVA treatment within 8 weeks prior to the study or were allergic to calcineurin inhibitors were excluded.

Participants who fulfilled the above criteria underwent a 1-week run-in period, during which their baseline dietary intake, emollient and drug use as well as information regarding their severity of AD were collected. Additional medications including their usual applications of topical CS or oral antihistamines were not permitted during the run-in and treatment period. They were then started on topical tacrolimus ointment twice daily on affected areas for 2 consecutive weeks. The clinical severity of AD was assessed with the SCORAD before run-in, at baseline level and the end of the treatment. The patients' and parents'/guardians' assessments of pruritus were also evaluated daily throughout the whole study period in the form of a written diary, with a visual analogue scale of 0 to 10, with 0 meaning "no-itch" and 10 meaning "worst itch imaginable". Nocturnal scratching of the subjects in both groups was evaluated by the Digitrac monitor at the first three consecutive days of baseline (days 8 to 10) and at three consecutive days after the 2-week treatment period (days 22 to 24). The purpose of this 'consecutive-day

measurement' was to ensure the reproducibility of data. Levels of TARC, CTACK, BDNF and SP were also detected using the previous ELISA techniques (Appendix II).

The degree of adherence to our study regimen was evaluated by weighing the amount of 'drugs' that remained in drug tubes, as well as additional drug usage as recorded by the patients (if any or due to severe exacerbation of the disease) and sleep disturbance in their Eczema Diary. Paired data on SCORAD, Digitrac data and laboratory marker levels before and after tacrolimus were analyzed two-tailed by Wilcoxon signed ranks test. *P*-values of less than 0.05 were considered to be significant.

3.4 Further application of Digitrac in pruritus of other medical fields

Pruritus is a common presenting complaint in many diseases other than AD (Gabriel and Crone, 2001). Therefore, this part of our study aimed to take a step forward in measuring nocturnal pruritus in non-atopic patients by recording their scratching behavior with Digitrac.

In particular, the study came across a 21-year-old pregnant woman presented with an itchy generalized bullous eruption with no past history of atopy or skin dryness (Figures 3.10 and 3.11). At week 19 of gestation, she developed an itchy rash over the limbs, which spread to the trunk. Blisters then appeared on the hands which subsequently also involved the feet. There were no fever, oral or genital lesions and she denied the use of over-the-counter or herbal medicine. Continuous pruritus and sleep loss due to the skin condition was reported by the patient.

On examination, she had multiple erythematous patches and plaques over the trunk and limbs. Bullae were seen on limbs, particularly on hands and feet. Ultrasonographic assessment revealed a single live fetus with normal anthropometric parameters and normal volume of amniotic fluid. A skin biopsy was performed (by the Dermatology Research Centre, The Chinese University of Hong Kong), which showed palmoplantar skin with eosinophilic spongiosis with superficial and mid interstitial inflammatory and eosinophilic infiltrates. Immunofluorescence study showed moderate staining for complement-3 (C3) at dermoepidermal junction. The collection of eosinophils at papillary dermis attempting to form subepidermal blister and the strong immunofluorescein stain for C3 favored the diagnosis of pemphigoid gestationis (PG).

The nocturnal scratching pattern was documented with the Digitrac wrist monitor on the day which the patient reported the most intense pruritis since her admission to the hospital. The wrist activities of a normal person and that of a patient with severe eczema were measured as reference.

Another rare case under my study of pruritus was a previously healthy 3-year-old girl who presented with a recent onset of generalized itch over her genitalia without rash in May 2005. She was treated as vulvo-vaginitis by a local general practitioner. There was no improvement in her symptoms, and itchiness worsened to affect the whole body. She was then treated as scabies by another doctor at that moment because there were red spots at the fingers. This little patient was then treated as eczema upon the consultation by another dermatologist, even though she and her family had no atopies and her skin did not show any typical pattern of skin involvement for AD. Treatment with various antihistamines including certirizine did not improve her skin condition. She subsequently developed antalgic gait with right hip pain (for 10 days) and persistent fever with cough and running nose a month later. The range of movement of her right hip was found to be decreased, and subsequent X-ray showed multiple osteolytic lesions with pathological fracture at right femur neck. Further imaging showed lesions involving right skull, right anterior fourth rib and left sacrum. Bone biopsy was performed in late June 2005, which showed small bone cell tumor with pathological fracture. Trephine examination performed a week later revealed abnormal lymphoid infiltration with medium to large lymphoid cells showing irregular nuclei and infiltration of small T-lymphocytes. The diagnosis of peripheral T-cell lymphoma was finally made with further radiological and

histopathological investigations, with persisting itch over right femur and complaint of nocturnal pruritus, affecting her sleep.

Despite the severe itch, onycolysis of the left index fingernail in this patient only showed minimal scratch marks on her right gluteal region with no flexural involvement. To further look at the intensities and extent of pruritus in such a rare disease, itch pattern prior to and following chemotherapy were documented by the Digitrac movement monitor. The results obtained were compared with nocturnal wrist movements of a normal subject and a child with severe AD.

Figure 3.1 Digitrac® movement recorder (IM Systems, Baltimore, MD)

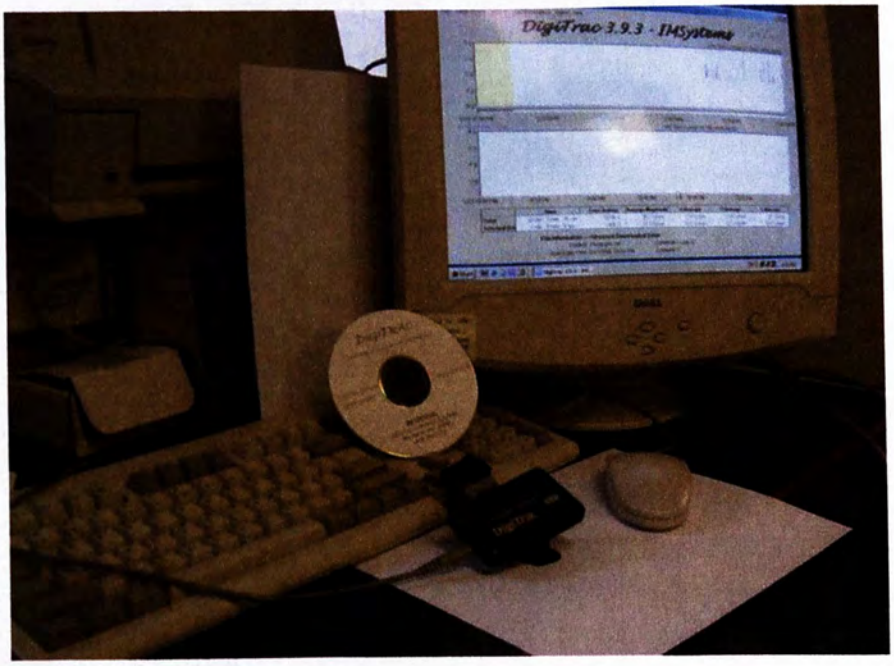


Figure 3.2 Digitrac3.9, A program facilitating downloading data stored inside Digitrac to computer for analysis (IM Systems, Baltimore, MD).

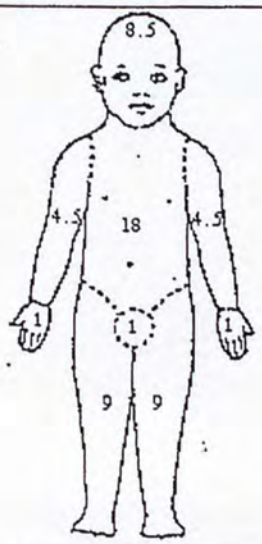
SCORAD
EUROPEAN TASK FORCE
ON ATOPIC DERMATITIS
(for children under 2 years)

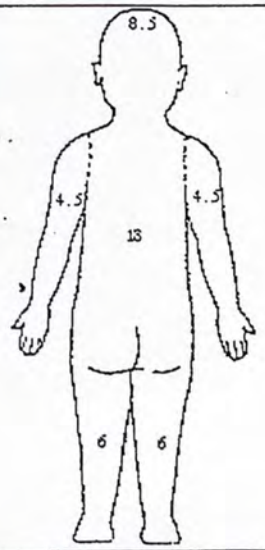
INSTITUTION

PHYSICIAN

Topical Steroid used
Name: _____
Amount / Month: _____(G)
Number of flares / Month: _____

Name: _____
Date of Birth: _____
Date of Visit: _____





A: EXTENT Please indicate the area involved _____

B: INTENSITY _____

CRITERIA	INTENSITY
Erythema	
Edema / Papulation	
Oozing / Crust	
Excoriation	
Lichenification	
Dryness *	

* Dryness is evaluated on uninvolved areas

MEANS OF CALATION
INTENSITY ITEMS
(average representative area)
0= absence
1= mild
2=moderate
3=severe

C: SUBJECTIVE SYMPTOMS PRURITUS + SLEEP LOSS

Visual analog scale (average for the last 3 days or nights)

PRURITUS (0 to 10) _____
0 10
SLEEP LOSS (0 to 10) _____

SCORAD A/5 + 7B/2 + C

TREATMENT:

REMARKS:

Figure 3.3 The SCORing Atopic Dermatitis index (SCORAD) diagnosis worksheet (1993)

a)

b)

Figure 3.4 Evaluation of Laboratory markers of AD and pruritus using enzyme-linked immunosorbent assay (ELISA) techniques.

Figure 3.5 Pentaherbs capsule. Patients were instructed to take 3 capsules twice daily. Capsules were considered to eliminate the taste of bitterness of traditional Chinese herbs.

CHILDREN'S DERMATOLOGY LIFE QUALITY INDEX

Hospital No:
Name: Diagnosis: CDLQI SCORE: ☐
Age:
Address: Date:

The aim of this questionnaire is to measure how much your skin problem has affected you OVER THE LAST WEEK. Please tick ✓ one box for each question.

1.	Over the last week, how **itchy, "scratchy", sore** or **painful** has your skin been?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
2.	Over the last week, how **embarrassed** or **self conscious, upset** or **sad** have you been because of your skin?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
3.	Over the last week, how much has your skin affected your **friendships**?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
4.	Over the last week, how much have you changed or worn **different** or **special clothes/shoes** because of your skin?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
5.	Over the last week, how much has your skin trouble affected **going out, playing,** or **doing hobbies**?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
6.	Over the last week, how much have you avoided **swimming** or **other sports** because of your skin trouble?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
7.	Last week, was it **school time?** → **If school time**: Over the last week, how much did your skin affect your **school work**?	Prevented school Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐ ☐
	OR		
	was it **holiday time?** → **If holiday time**: How much over the last week, has your skin problem interfered with your enjoyment of the **holiday?**	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
8.	Over the last week, how much trouble have you had because of your skin with other people **calling you names, teasing, bullying, asking questions** or **avoiding you**?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
9.	Over the last week, how much has your **sleep** been affected by your skin problem?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐
10.	Over the last week, how much of a problem has the **treatment** for your skin been?	Very much Quite a lot Only a little Not at all	☐ ☐ ☐ ☐

Please check that you have answered EVERY question. Thank you.

Figure 3.6 The Children's Dermatology Life Quality Index (CDLQI) (Lewis-Jones and Finlay, 1995)

兒童皮膚科生活質素指數

呢份問卷調查嘅目的 量度過去一個星期裏面，你嘅皮膚問題對你生活嘅影響有幾大。請你喺每一條問題嘅其中一個空格畫一個剔號。

1. 喺過去一個星期裏面，你嘅皮膚痕、癢、酸痛或者痛嘅程度點樣呢？	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐
2. 喺過去一個星期裏面，你因爲皮膚問題而產生尷尬、太注意自己、唔開心或者傷心嘅程度點樣呢？	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐
3. 喺過去一個星期裏面，你嘅皮膚問題對你同朋友之間嘅關係影響有幾大？	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐
4. 喺過去一個星期裏面，你嘅皮膚問題令你換衫／鞋或者着特別嘅衫／鞋多唔多呢？	非常多	☐
	多	☐
	些少	☐
	無	☐
5. 喺過去一個星期裏面，你嘅皮膚問題對你出街、玩或者做你鍾意做嘅嘢嘅影響有幾大？	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐
6. 喺過去一個星期裏面，你因爲皮膚問題而要避免游水或者做其他運動嘅情況多唔多？	非常多	☐
	多	☐
	些少	☐
	無	☐
7. 上星期，係唔係返學時間 / 或者 / 係唔係假期 — 如果係返學時間：喺過去一個星期裏面，你嘅皮膚問題對你嘅學業有幾大影響？	不能上課	☐
	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐
如果係假期：喺過去一個星期裏面，你嘅皮膚問題妨礙你享受假期有幾嚴重？	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐
8. 喺過去一個星期裏面，你因爲皮膚問題而俾人叫花名、笑、「蝦」、問問題或者避開你，咁嘅麻煩多唔多呢？	非常多	☐
	多	☐
	些少	☐
	無	☐
9. 喺過去一個星期裏面，你嘅皮膚問題影響你瞓覺多唔多呢？	非常多	☐
	多	☐
	些少	☐
	無	☐
10. 喺過去一個星期裏面，皮膚護理帶俾你嘅問題有幾大？	非常嚴重	☐
	嚴重	☐
	些少	☐
	無	☐

請你檢查你係唔係已經答晒所有問題。多謝。

Figure 3.7 The Cantonese-translated version of the CDLQI (Chuh, 2003)

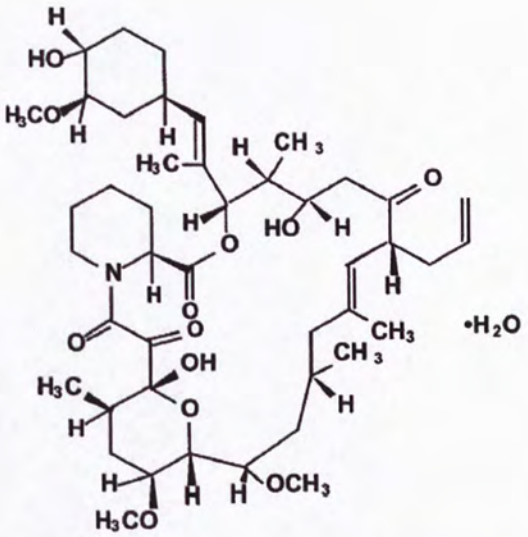


Figure 3.8 Structure of tacrolimus. The CAS Registry name of tacrolimus is as follows: [3S-[3R*[E(1S*, 3S*, 4S*)], 4S*, 5R*, 8S*, 9E, 12R*, 14R*, 15S*, 16R*, 18S*, 19S*, 26aR*]]-5,6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 24, 25, 26, 26a-hexadecahydro-5, 19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy 4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate. The empirical formula of tacrolimus is C44H69NO12H2O; formula weight is 822.05 daltons.

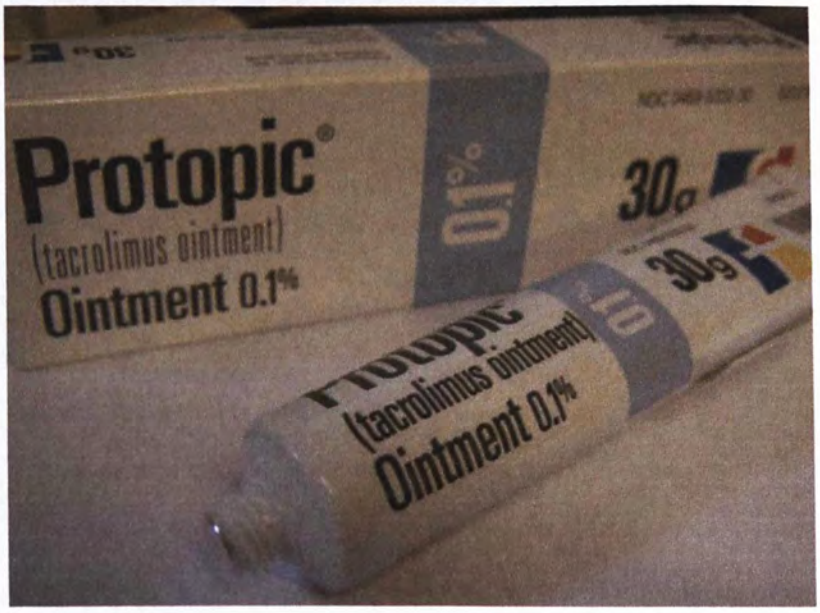


Figure 3.9 Tacrolimus ointment

Figure 3.10 Wide spread bullous lesions with erythematous base found on the trunk on the patient suffering from pemphigoid gestationis.

Figure 3.11 Hemorrhagic bullae on the palm of the patient suffering from pemphigoid gestationis.

Chapter 4

Results and Discussions

4.1 Digitrac validation

4.1.1 General demographic background data

43 patients (24 males, 19 females) and 37 healthy controls (14 males, 13 females) with no atopy history were recruited for the validation of Digitrac during the period August 2004 to August 2005. The mean (standard deviation) age for patients and controls was 11.9 (3.6) years and 11.9 (3.4) years, respectively (p=0.975). The mean (standard error of mean, SEM) SCORAD in the group of AD patients was 49.0 (3.43). All subjects were enrolled into study with a SCORAD score of ≥15. The controls had either trivial skin diseases (2 with acne, 1 with alopecia totalis, and 1 with dandruff) or were normal healthy children of the clinic staff.

The mean (SEM) serum total IgE level and eosinophil percentage in PB were 5844 (1293) kIU ml^{-1} and 8.4 (0.8) %, respectively. As both means are higher than the suggested reference value of 180 kIU ml^{-1} and 6%, the patients showed signs of atopy.

Although SCORAD was the gold standard used in this study, it did not contain a severity grading and the scoring of scratching and sleep disturbance lacked objectivity and were difficult to be studied. Since the main objective is to validate the objectiveness of the device, a score of objective SCORAD, i.e. the total SCORAD score minus subjective pruritus and sleep loss scores reported by parents, was also included in statistical analysis during device validation. The objective SCORAD score has rid on subjective symptoms and provides a severity grading, with score < 15 considered to be

mild, 15 ≤ score ≤ 40 to be moderate, and > 40 to be defined as severe AD (Kunz *et al.*, 1997).

It was hypothesized that the patients would find themselves difficult to get asleep at the early hours after getting to bed. Therefore, this study focused on the first few hours of sleep for the first part of validation. To define the beginning of sleep, the patients are required to give a verbal report of the time they got to bed. When visually observed and compared with controls with the aid of the computer program, most wrist activities in both groups occurred in the first three hours of bed resting (Figure 4.1). The average wrist activities in the early hours of sleep of both patients and controls were shown in table 4.1. Activities of individual hour within the first three hours of sleep of AD patients showed significant difference with that of the controls, after performing Wilcoxon sign-ranked test, with $p<0.01$ (Table 4.1).

4.1.2 Wrist activities

By performing Pearson parametric correlations, the average wrist activities of the second and third hour of sleep correlated well with overall and objective SCORAD ($p=0.001$ for second hour, $p<0.001$ for third hour of sleep) (Table 4.3). Similar correlations were also observed for the average value for the first three hours of sleep ($p<0.001$), yet could not be found in the first hour. There were also significant correlations between average activities and component scores of SCORAD (Table 4.3), with the highest correlation of $\rho=0.590$ and $=0.551$, $p < 0.001$ for overall extent and intensity, respectively. However, there was no correlation with pruritus score within the SCORAD, which was subjectively reported by the patients or their parents.

The frequencies of limb motions during early hours of sleep were also recorded by the device during validation. Wrist activities at frequencies of 0 to 3 hertz (Hz, or cycles per second) consisted of the largest portion of activities (Figure 4.2 and Table 4.2), suggesting the nature of wrist movements of the group of AD patients in this study were of small amplitude. By performing Pearson parametric correlations, all frequency-specific wrist activities at 0-1 Hz, 1-2 Hz, 2-3 Hz and a total of 0-3 Hz correlated well with total and objective SCORAD (p=0.005, 0.001, 0.001 and 0.002 for SCORAD, 0.004, 0.001, 0.002 and 0.002 for objective SCORAD, respectively) (Table 4.4). Similar to average activity, there were no correlations between Hz-specific wrist activities and pruritus score.

4.1.3 Laboratory markers and factor analysis

The mean (SEM) levels of TARC and CTACK were 760 (180) and 688 (119) pg/ml, respectively. TARC revealed a strong correlation with Digitrac findings, after performing Pearson correlation test (Table 4.5). Wrist activities of the second hour, third hour and an average of 0-3 hour of sleep correlated well with plasma TARC concentrations, but not with CTACK. Such a correlation could not be found for the data during the first hour of sleep. Frequency-specific wrist activities also showed sounding correlations with TARC levels.

When the interdependence among subjective and objective severity scores, Digitrac readings and laboratory parameters were assessed for factor analysis, Barlett's test of sphericity indicated a correlation between these variables because the correlation matrix was statistically different from an identity matrix (χ^2 = 366.82, degree of freedom

= 28, $p<0.00001$) (Table 4.6). The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.630, which was above minimum acceptable value, indicating that the data was likely to factor well. Factor analysis yielded four factors that could explain 81.37% of the total variance in the data set (Table 4.6). In brief, the objective indicators (SCORAD, objective SCORAD and related components within), self-reported scores (subjective components of SCORAD including pruritus and sleep loss), Digitrac findings and objective laboratory markers (Plasma IgE concentrations and total white cell count) were separate domains during assessment of AD in the validation study, indicating their independent importance for interpretation of data.

4.1.4 Interpretation of results

Wrist movements did not correlate with the subjective symptoms of pruritus. Previous studies documented that the subjective symptoms were not necessarily correlated with the objective index of disease severity of AD in patients (Hon *et al.*, 2003). This validation study further speculated that it is either the subjective symptoms of pruritus reported by parents are imprecise, or mechanisms other than disease extent or severity, that is responsible for the pathogenesis of these symptoms. As previously reported, it is common for children in Hong Kong to be looked after by a maid and therefore parents may not know or witness their child's condition at night. Some older children may have their own room and therefore the quality of their sleep is not witnessed. Possible alternative explanation is that pruritus may be due to mechanism other than the extent and intensity of skin inflammation. Scratching and sleep disturbance has been difficult to study (Rees and Laidlaw, 1999). Some scoring systems, therefore, have

bypassed these assessments and adopted a more objective approach. The typical example is the objective SCORAD, an index without the pruritus and sleep-loss components. However, itch and scratching should never be neglected, and are important parameters to be assessed objectively in order (i) to document the amount of disability or impairment of quality of life caused by the disease as well as (ii) to gauge therapeutic efficacy of alternative, expensive or potentially harmful treatment. Therefore the validation of Digitrac might provide an alternative for measuring these important clinical parameters.

AD is a very common disease in children and is studied at various levels by physicians. For instance, when referring to pruritus and sleep quality, general practitioners or paediatricians might be interested in linking up with the quality of life of children with AD. Dermatologists, on the other hand, may be interested in the advances in therapeutics and specific clinical scores, whereas immunologists may be interested in chemokine responses instead. Assessment of wrist movements appears to be an inexpensive and noninvasive methodology that generates easy-to-comprehend data which are correlated all around, and hence provide a platform, for linking up various markers and scores.

It is important to ask the fundamental question as to what is more important to measure, itch or scratching? Although unbearable to the sufferer at times, the sensation or feeling of itch might or might not be totally translated to scratching and restless wrist movements. Itch by itself does not lead to further damage or inflammation. Yet as previously discussed, itch and scratch were two interdependent parameters and researchers have proven strong correlations among pruritus and sleep loss.

It is also important to understand what is being measured when one measures AD. Scratching is important in the pathogenesis of AD, causing further breakage of skin barrier and release of inflammatory cytokines. Various studies recently reported a significant correlation between serum CTACK concentration and overall SCORAD (ρ=0.394, p=0.016) and its extent (ρ=0.528, p=0.001) and intensity components (ρ=0.429, p=0.008); but not with subjective symptoms of sleep loss and pruritus (Hon *et al.*, 2004a). All these suggested that measurements of objective laboratory markers are useful to have a more complete look at the actual picture of pathogenesis of pruritus and wrist movements. It is therefore not surprising that our study demonstrates a significant correlation between TARC and wrist movements rather than itch. Our findings concur with the observation that TARC, being a key immunological marker, correlates with objective signs (extent and intensity) but not subjective features (pruritus) of AD. Wrist movements, but not the subjective symptom of itch, do appear to have an immunological basis in terms of pathogenesis.

To date, there has been no perfect gold standard for assessing AD severity. Worse still, there has been no objective gold standard for assessing the inter-relationship between pruritus and sleep loss in children. It has been found out that total sleep efficiency was lower in patients with severe AD than that obtained in the control (median: 72% versus 88%; p = 0.039) (Hon *et al.*, 2005b). However, measurement of sleep efficiency was cumbersome and required hospitalization for overnight assessment. Other investigators have suggested that wrist movements were also correlated with sleep efficiency and accelerometer device might be an option to be used at patient's own home (Benjamin *et al.*, 2004). Quantifying new markers and correlating their levels with

clinical scores may provide better understanding of AD and facilitate researches to improve its management.

4.1.5 Drawbacks of the validation

There are several limitations in this study. Firstly, only the nocturnal wrist movements of the dominant hand were recorded. Ideally, limb movements of all four limbs should be recorded. As correlations for movements in various limbs are good according to other studies, they also suggest that little information would be lost if only one limb was measured.

Another important ambiguity that should not be neglected is that the recorded limb movements might not be solely due to scratching. It could be non-specific restless movements, which could not be delineated without video analysis (Benjamin *et al.*, 2004). Benjamin *et al.*, using the ActiGraph, demonstrated that scratching and restless movements were highly correlated with each other and with video results. They also pointed out that little is gained to delineate these movements one from the other. It may be that the subjective feeling of itch leads to both purposeful scratching activities and purposeless restless movements. The validation study this time therefore did not assess individual movements but evaluated the frequencies and timing of assessment of these movements instead. Based on the fact that wrist activities were concentrated on low-frequency bandwidth, other non-specific movements that fall out of the range were further excluded for study. Further, the study demonstrates that only very little movements, be it scratching or restless movements, occur in normal control subjects with a very strong basis of significant difference from AD subjects. Therefore, a large portion

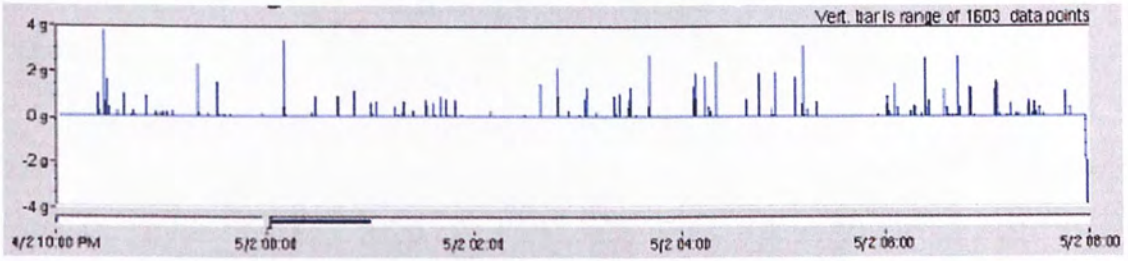
of wrist movements are AD-associated, and is not necessary to segregate one form of movements from the other for the purpose of analysis.

Lastly, we did not monitor the wrist movements for longer period, which might cause a "first-night effect" leading to inaccuracies of the measurement. In the later part of the study, continuous measurements were performed to counteract with this shortcoming.

4.1.6 Summary

To summarize, wrist movement is easy to be documented non-invasively at the patient's own home, which is the most comfortable and appropriate environment for validation study. Its clinical nature and basis is objective and correlated with clinical laboratory markers of eczema severity. Although wrist movement monitoring is not going to replace clinical or biochemical assessment, it offers supplementary objective data to the understanding of the complex itch-scratch cycle in children with AD and thus worth further explorations and applications in various clinical trials. From the above results, wrist activities between 0 and 3 Hz for first three hours of sleep might become a useful non-invasive home-based objective indicator of sleep disturbance and eczema severity in children. The findings that wrist activities were correlated with wrist movements were consistent with the findings reported with various studies using accelerometer devices (Benjamin *et al.*, 2004). In addition, with reference to the relationship between wrist activities, pruritus and slower movements (0 – 3 Hz) and related laboratory markers, the validation accurately documented that it is the occurrence of these movements during early sleep rather than itching that are T-helper-2 chemokine mediated.

a)



b)

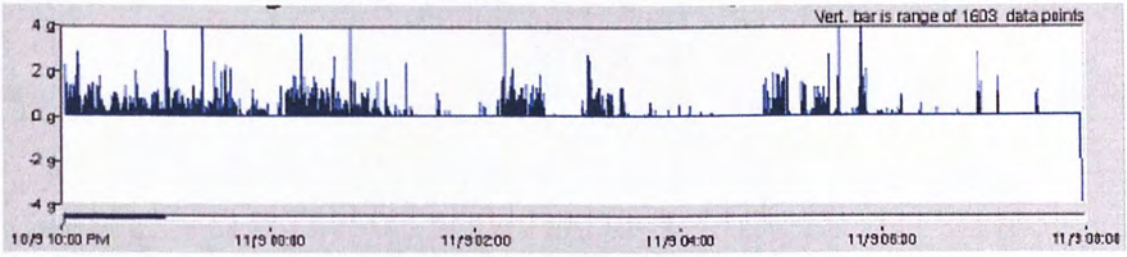
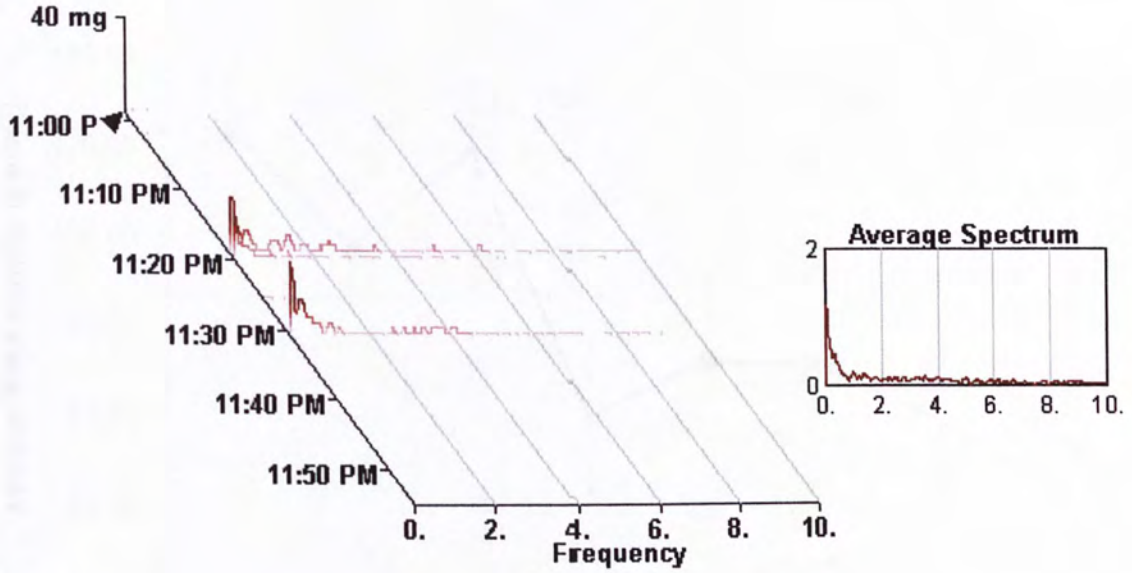


Figure 4.1 Visualizations of wrist activities during sleeping in (a) a normal subject versus (b) a patient with severe atopic dermatitis with the aid of Digitrac 3.9 (IM Systems, Baltimore, MD). Data were expressed in units of g min^{-1} within each individual hour. Most wrist activities occurred in the first three hours during sleep.

a)



b)

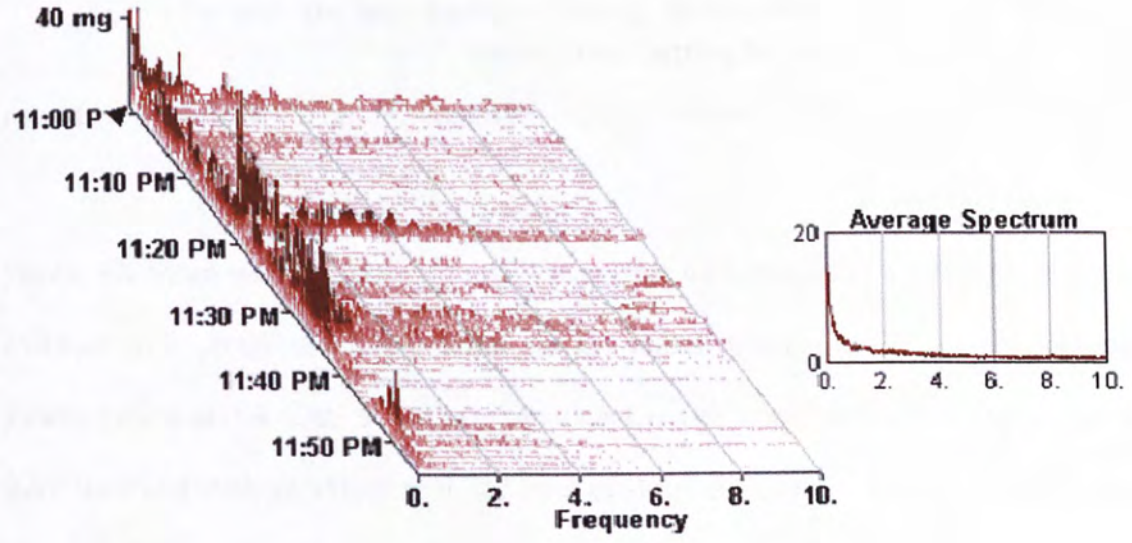
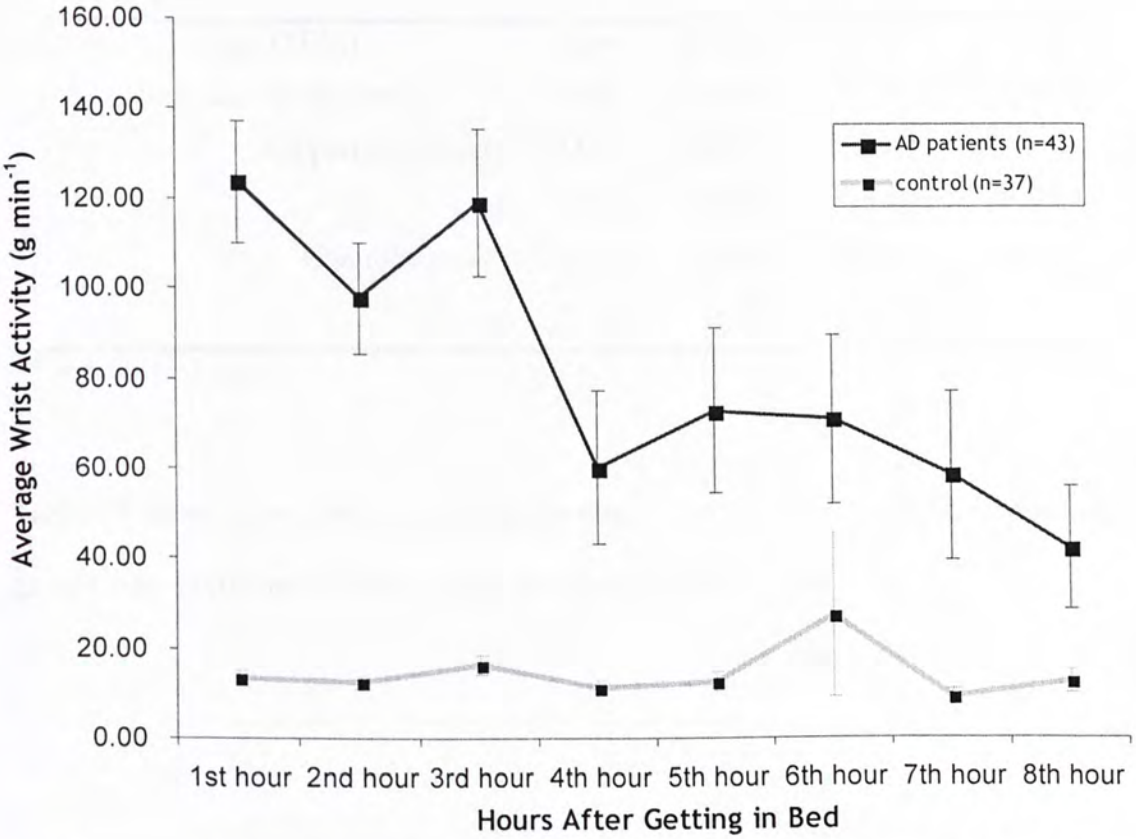


Figure 4.2 Typical changes in frequencies of wrist activities in a) a normal control, versus b) a child with severe atopic dermatitis in the early zero-to-three hours of sleep. Most activities occurred in the frequency bandwidth 0-3 Hz.



p-value	0.154	0.001**	<0.001**	0.172	0.069	0.500	0.217	0.740

Figure 4.3 Mean wrist activities (g min^{-1}) over time for consecutive 8 hours of sleep for children with severe atopic dermatitis (n=43) versus controls (n=37). By performing Pearson correlations with SCORAD, significant results were obtained at second hour, third hour and average values from 0-3 hour of sleep. As a result, analysis onwards was based on findings during early hours of sleep.

Mean (SEM) wrist activity (g min^{-1})	First hour	Second hour	Third hour	Average 0-3 hour
AD patients (n=43)	**123.6****	**97.8****	**118.9****	**113.4****
	(13.6)	(12.3)	(16.4)	(10.2)
Controls (n=37)	**33.0**	**32.2**	**37.8**	**34.1**
	(1.6)	(1.4)	(2.2)	(1.2)

** $p < 0.01$; 2-tailed.

Table 4.1 Mean wrist activity for AD patients and controls (g min^{-1}). Difference among 2 groups was significant for every single hour over 0-3 hour.

Mean (SEM) Hz-specific wrist activity (mg min^{-1})	0-1 Hz	0-2 Hz	0-3Hz	Total 0-3 Hz
AD patients (n=43)	269.6	223.1	172.4	665.0
	(29.9)	(22.5)	(17.0)	(67.7)

Table 4.2 Frequency-specific mean wrist activity of AD patients for the first three hours of sleep (mg min^{-1}).

Average wrist activity ($g\ min^{-1}$) (n=43)		First hour of sleep	Second hour of sleep	Third hour of sleep	Average 0-3 hours
SCORAD	Pearson ρ	0.221	.473(**)	.521(**)	.569(**)
	p	0.154	.001	.000	.000
Objective		0.206	.502(**)	.521(**)	.574(**)
SCORAD		0.186	.001	.000	.000
Extent		0.177	.519(**)	.562(**)	.590(**)
		0.257	.000	.000	.000
Intensity		0.207	.482(**)	.493(**)	.551(**)
		0.183	.001	.001	.000
Erythema		0.154	.506(**)	.464(**)	.522(**)
		0.324	.001	.002	.000
Edema /		0.170	.403(**)	.456(**)	.483(**)
Papulation		0.277	.007	.002	.001
Oozing /		0.290	.161	.311(*)	.361(*)
Crusting		0.060	.302	.043	.018
Excoriation		0.249	.254	.443(**)	.451(**)
		0.107	.100	.003	.002
Lichenification		0.037	.463(**)	.423(**)	.430(**)
		0.813	.002	.005	.004
Dryness		0.088	.462(**)	.245	.357(*)
		0.574	.002	.114	.019
Subjective score		0.217	.218	.366(*)	.381(*)
		0.163	.159	.016	.012
Pruritus		**0.115**	**.183**	**.314(*)**	**.294**
		0.461	**.240**	**.040**	**.056**
Sleep loss		0.270	.218	.359(*)	.401(**)
		0.080	.160	.018	.008

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4.3 Correlations of Digitrac findings of average wrist activity ($g\ min^{-1}$) with SCORAD, objective SCORAD and related component scores. All data were expressed in Pearson's ρ followed by a 2-tailed *p*-value and so on.

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Frequency-specific Average wrist activity (mg min^{-1}) (n=43)		0-1 Hz of limb motions	1-2 Hz of limb motions	2-3 Hz of limb motions	Total 0-3 Hz
SCORAD	Pearson ρ	0.419**	0.485**	0.473**	0.465**
	p	0.005	0.001	0.001	0.002
Objective		0.427**	0.474**	0.461**	0.462**
SCORAD		0.004	0.001	0.002	0.002
Extent		0.436**	0.492**	0.473**	0.475**
		0.003	0.001	0.001	0.001
Intensity		0.415**	0.456**	0.446**	0.446**
		0.006	.002	0.003	0.003
Erythema		0.425**	0.421**	0.394**	0.426**
		0.004	0.005	0.009	0.004
Edema /		0.327*	0.373*	0.384*	0.365*
Papulation		0.032	0.014	0.011	0.016
Oozing /		0.318*	0.391**	0.368*	0.363*
Crusting		0.037	0.010	0.015	0.017
Excoriation		0.269	0.326*	0.328*	0.309*
		0.081	0.033	0.032	0.044
Lichenification		0.308*	0.322*	0.334*	0.327*
		0.044	0.035	0.029	0.032
Dryness		0.289	0.309*	0.290	0.303*
		0.060	0.044	0.059	0.048
Subjective score		0.262	0.384*	0.381*	0.339*
		0.090	0.011	0.012	0.026
Pruritus		**0.157**	**0.249**	**0.249**	**0.214**
		0.316	**0.108**	**0.108**	**0.168**
Sleep loss		0.313*	0.442**	0.437**	0.395**
		0.041	0.003	0.003	0.009

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 4.4 Correlations of Digitrac findings of frequency-specific average wrist activity (mg min^{-1}) with SCORAD, objective SCORAD and related component scores. All data were expressed in Pearson's ρ followed by a 2-tailed *p*-value.

	Mean (SEM) Level in plasma		Average wrist activity (g min^{-1})				Frequency-specific wrist activity (mg min^{-1})			
			First hour	Second hour	Third hour	Average 0-3 hour	0-1 Hz	1-2 Hz	2-3 Hz	Average 0-3 Hz
TARC	759.78	Pearson ρ	0.259	**0.434****	**0.396***	**0.489****	**0.391***	**0.413***	**0.481****	**0.428****
	(180.35)	*p*	0.121	**0.007**	**0.015**	**0.002**	**0.017**	**0.011**	**0.003**	**0.008**
CTACK	688.28	Pearson ρ	0.103	0.054	0.095	0.070	0.069	0.134	0.069	0.092
	(118.71)	*p*	0.545	0.750	0.574	0.681	0.684	0.429	0.686	0.588

** $p < 0.01$, * $p < 0.05$; 2-tailed.

Table 4.5 Correlations between TRAC, CTACK and average wrist activity over the first three hours of sleep.

	Factor 1	Factor 2	Factor 3	Factor 4
SCORAD	**0.783**	0.421	0.282	0.350
Objective SCORAD	**0.852**	0.281	0.285	0.327
Extent	**0.772**	0.358	0.294	0.288
Intensity	**0.859**	0.242	0.273	0.335
Erythema	**0.831**	0.207	0.224	0.027
Edema/Papulation	**0.700**	0.133	0.247	0.534
Oozing/Crusting	0.231	0.189	0.430	**0.575**
Excoriation	**0.680**	0.145	0.202	0.460
Lichenification	**0.820**	0.078	0.172	0.068
Dryness	**0.814**	0.373	0.010	-0.016
Subjective Score	0.266	**0.864**	0.188	0.339
Pruritus	0.400	**0.753**	0.037	0.295
Sleep	0.120	**0.838**	0.286	0.330
Average wrist activity for 0-3 hours of sleep	0.342	0.141	**0.887**	0.010
0-3Hz-specigic activity for 0-3 hours of sleep	0.206	0.152	**0.928**	-0.022
Plasma IgE concentration	0.434	0.301	-0.023	**0.619**
Peripheral blood eosinophil count	0.499	**0.590**	0.050	-0.212
Total blood White cell count	0.024	0.141	-0.111	**0.639**
Eigenvalue	6.55	3.22	2.47	2.41
Total variance explained, %	36.37	17.91	13.74	13.37

Table 4.6 Varimax rotated factor-loading matrix for all 43 AD patients. Bold values represent the highest factor loadings. The objective indicators (SCORAD, objective SCORAD and related components within), self-reported scores (subjective components of SCORAD including pruritus and sleep loss), Digitrac findings and objective laboratory markers (Plasma IgE concentrations and total white cell count) were separate domains during assessment of AD in the validation study, indicating their independent importance for interpretation of data.

4.2 Digitrac in traditional Chinese herbal medication clinical trial

4.2.1 General information

28 have completed the whole TCM course with Digitrac monitoring and provided adequate data for analysis, None of them violated the patient selection criteria. All subjects complied well with the instructions given on consuming the medication, without difficulties for capsule swallowing or any other severe adverse effects or acute exacerbation of eczema according to the returned eczema diary. For the device, all patients followed instructions clearly for using the device and none of them reported any discomfort throughout the process. The patients' starting and ending time of sleep were recorded clearly by the parents. Only those wrist movement data collected afterwards were considered to be valid.

To ensure safety, liver and renal function tests have been performed at the beginning and at the end of the study. None of the participants showed any abnormality. Mean (SEM) serum total IgE, PB eosinophil and total white cell counts before and after the 12-week trial medication were 5413 (1391) vs. 4747 (1256) kIU ml^{-1}, 8.3 (1.0) vs. 8.3 (1.0) and 7.1(0.4) vs. 7.8 (0.4) $x10^{9}$ L^{-1}, respectively. While IgE and eosinophils showed a decreasing trend, the difference was not significant after performing paired-samples t-test.

4.2.2 SCORAD, wrist activities and CDLQI

The mean (SEM) SCORAD scores of the patients before and after trial were 46.2 (3.4) and 42.4 (3.7) respectively (Table 4.7). Objective SCORAD scores, after excluding the subjective scoring, were 36.1 (2.9) and 33.3 (2.9) before and after trial, respectively. The difference between both SCORAD and objective SCORAD were not significant. The score on sleep loss reported by parents, however, showed statistically significant difference among each visit (p=0.042) (Table 4.7).

All patients (or parents / caregivers, provided if the patient was too young to understand the wordings of the questionnaire) were required to fill in the CDLQI questionnaire prior to the study, at the baseline of the trial. Minimum instructions were given; therefore the CDLQI score would be able to solely represent the subjective perception of the impairment of quality of life by the patients or parents themselves, without interference from investigators. The mean (SEM) total CDLQI score of the patients right before starting of trial was 8.79 (1.01). Correlations of individual question scores with subjective symptoms scores within the SCORAD were performed (Table 4.8). Subjective scores, including pruritus and sleep loss, correlated well with the total CDLQI score (p<0.01) and also scores of the questions related to scratching and sleep loss (p<0.05). Such correlations were probably due to the nature of subjectiveness of both scoring systems. Similar statistical tests were also performed with baseline readings from Digitrac and the data from the device did not correlate significantly with CDLQI outcomes, except the average activity with total CDLQI score (ρ=0.440, p<0.05).

Patients were instructed to wear Digitrac on the first day and the last day of the study and pre-post Digitrac data were compared using paired t-tests (Figure 4.4). The

mean (SEM) wrist activity of the patients over the first 3 hours decreased significantly from 113.4 (12.2) g min^{-1} to 76.2 (8.1) g min^{-1} before and after TCM trial (p=0.001) (Table 4.9a). When taking individual hours into considerations, significant difference was also obtained for the second and third hour of sleep of the patients after receiving the 12-week TCM medication. Wrist activity of 0-3 Hz within the period also showed significant decrease, from 689.4 (84.5) mg min^{-1} to 410.3 (50.0) mg min^{-1} (p=0.002) (Table 4.9b).

For ensuring reproducibility of results from previous validation, the whole set of Digitrac data in TCM trial were also compared back with the SCORAD and related component scores (n=56) (Table 4.10). After performing Pearson correlations, data recorded from Digitrac strongly correlated with total SCORAD, objective SCORAD, extent and intensity, (p=0.007, 0.005, 0.003 and 0.008, respectively) but not with subjectively reported scores of pruritus and sleep loss (p=0.062, 0.079, respectively).

4.2.3 Laboratory findings

Swabs were taken obtained from the anterior nares, flexures (anterior neck, antecubital fossae and popliteal fossae) and the worst inflammatory skin areas of the patients before and after TCM trial. The most severely affected region from each patient during separate visits was taken for statistical analysis. Among the 28 patients, 12 showed improvements in *Staphylococcus aureus* colonization on their most severely affected region, 7 subjects deterioration and 9 with no change. The difference was not statistically significant, indicating TCM might not be useful in improving AD through elimination of *S. Aureus.*

The levels of various laboratory markers before and after TCM trial were obtained through ELISA assays. The mean (SEM) levels of the two inflammatory chemokines, plasma TARC and CTACK, before and after trial were 824 (189) vs. 492 (101) and 1424 (136) vs. 1087 (66) pg/ml, respectively. Both levels were significantly decreased (p=0.013, 0.020, respectively) (Table 4.11). For pruritic markers, both levels of plasma brain-derived neurotrophic factor (BDNF) and substance-P (SP) dropped significantly, from 1798 (177) to 1378 (146) for BDNF, and 93.6 (8.0) to 72.9 (6.4) pg ml^{-1} for SP, respectively (p=0.002). Pearson correlations of BDNF and SP were done against subjective symptom scores of SCORAD and readings from Digitrac (n=56) (Table 4.12). The markers correlated very well with the Digitrac data (Pearson ρ=0.878 and 0.897 for BDNF and SP respectively, p<0.00001), but not significantly correlated with the subjective report scores from patients or parents.

4.2.4 Interpretation of results

The study showed that Pentaherbs capsules apparently improved the severity of AD in these patients, yet not significant. The actual therapeutic effects appeared to persist along the whole treatment course, yet by referring to SCORAD score, the extent of apparent improvement was slow.

In Chinese medicine, "wind", "dampness" and "heat" are considered to be the main pathogenic factors. The five herbs used in the study of this formulation are proposed to work as follow: *Jingyinhua* and *Bohe* clear wind-heat from the exterior, *Danpi* clears heat from blood, while *Cangzhu* and *Huangbai* clear the wind-heat from interior. This formula has been extensively used in China for treatment of allergic diseases including

eczema, asthma and allergic rhinitis. Pharmacological studies have documented that these herbs have anti-allergic, anti-inflammatory and sedative action for itch and they have been previously used in the study of childhood AD (Xu *et al.*, 1997;Huang, 1998).

The actual action mechanism of pruritus improvement of sleep loss or pruritus of traditional Chinese medication still leaves a large room for further research. Currently only little has been done on related research field, with only one conducted using a TCM called *Sho-seiryu-to* marketed in Japan (Sakaguchi *et al.*, 1997), which claimed to be an antihistamine / anti-allergy medication, inhibiting vascular permeability induced by histamine and histamine release by mast cells, hence soothing the sensation of pruritus. Our study took a step forward by applying devices to monitor improvement in an objective manner, thus ensuring the efficacy of medications and providing an insight into the mechanism of the drugs in its anti-inflammatory and anti-pruritic roles, basing on the significant differences in the laboratory marker levels. In particular, the strong correlations of pruritic markers with Digitrac and the poor correlations with subjective scores in this part of the study were important as they gave good rationale for the anti-pruritic effect of this alternative medication as well as emphasizing the basis in objectiveness of different measures.

However, the findings for *S. Aureus* of the trial indicated TCM might not be strong enough to suppress the acute inflammations caused by this AD-associated bacterium. Much work is still necessary to be done towards this aspect.

4.2.5 Safety of TCM use

The approach of herb selection has to be very careful as adverse effects of Chinese herbal medicines have been reported (Perharic-Walton and Murray, 1992;Ferguson *et al.*, 1997;Fung *et al.*, 1999). It is even more dangerous for practitioners to bear a misconception that TCM are "natural" substances and should not pose any side effects (Koo and Desai, 2003). Last but not least, the safety concern is heightened by the fact that standardisation of preparation, prescription and dosage of TCM is still very poor and varies with the prescriptions of individual practitioners, thus hindering its research progress.

Based on safety emphasis, practitioners of TCM should be aware of the possible side effects with various TCM drugs and drug combinations that may occur, and greater caution should be exercised in their use (Koo and Desai, 2003). The Pentaherbs formula used in this trial might be too mild for Hong Kong AD patients to adapt for overcoming the local warm and moist climate, which are possible sources for AD worsening through the mechanism of alloknesis, thus only showing mild and gradual improvement throughout this part of the study. While the impact of such environmental factors vary among different regions, other herbs such as *Cortex Moutan Radix*, Radix *Paeoniae alba, Potentilla chinensis ser* and *Radix glycyrrhizae* were also commonly used in eczematous conditions (Zhang, 1990) and should be considered for trials of TCM in the long run. Earlier studies also noted that *Clematis armandii*, *Lophatherum gracile*, *Dictamnus dasycarpus*, *Tribulus terrestris* and *Schizonepeta tenuifolia* had been shown to be beneficial and safe for the treatment of severe AD in both children (Sheehan and Atherton, 1994).

In Hong Kong, a controlled trial of Zemaphyte (a standardized formulation containing ten Chinese herbs) was conducted on 37 Chinese patients with recalcitrant AD for 8 weeks. No beneficial or adverse biochemical effects were demonstrated (Fung *et al.*, 1999). None of these herbs have pharmacological evidence regarding anti-allergic and anti-inflammatory effects. *Clematis armandi* used in Sheehan and Atherton's studies has diuretic effect and its long-term use necessitates potassium supplementation to avoid hypokalaemia. This herb was not used in our study in view of this safety concern.

4.2.6 Summary

To summarize, treatment with the Pentaherbs formula did not result in the hypothesized therapeutic effect, yet still remained useful as a supplementary medication in aiding acute inflammatory condition and sleeping, according to the findings. This study was limited by the relatively small sample size. Nevertheless, the clinical effects of the Pentaherbs capsules, and in particular, its usage in QoL improvement for AD, appeared to be impressive. TCM was appealing in aiding the patient to sleep better after a three-month intake, according to the Digitrac findings. The objectiveness of the device was further proved by its poor correlations with the scores from CDLQI, a subjectively-based questionnaire to be filled in. However, the correlation of average activity with the total score might propose the role of Digitrac in assessing extent of QoL impairment in AD patients. In order to look for improvement of quality of life in depth, TCM trials in the future should consider using additional scoring scales, e.g. CDLQI, to aid related clinical investigations.

n=28	**Baseline mean (SEM)**	**6-week Follow-up mean (SEM)**	**12-week Follow-up mean (SEM)**
SCORAD	**46.2 (3.4)**	**44.5 (7.5)**	**42.4 (3.7)**
objective SCORAD	**36.1 (2.9)**	**35.1 (5.9)**	**33.3 (2.9)**
Extent	42.9 (3.9)	42.9 (7.3)	40.5 (3.8)
Intensity	7.9 (0.6)	7.6 (1.3)	7.2 (0.7)
Subjective Score	10.1 (0.8)	9.3 (1.6)	9.1 (1.0)
Pruritus	5.43 (0.39)	5.17 (0.87)	5.17 (0.46)
Sleep Loss	**4.66*** (0.45)	**4.17*** (0.70)	**3.91*** (0.56)

* Difference is significant at the 0.05 level (2-tailed).

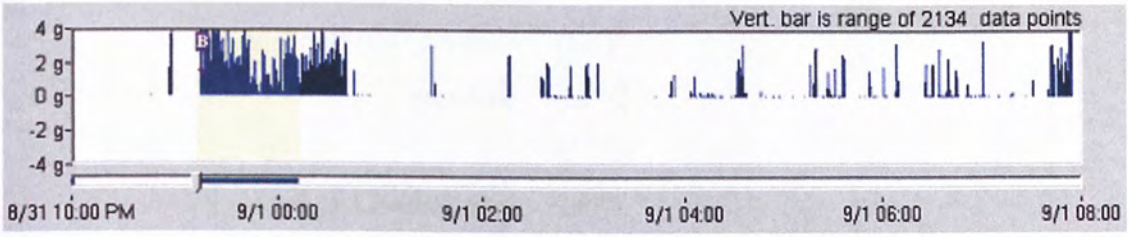
Table 4.7 Changes in the SCORAD and respective component scores of patients before and after traditional Chinese medication (TCM) clinical trial.

	Pearson coefficient, ρ (n=28)				
CDLQI components	**Scratching**	Clothes	**Sleep**	Treatment	**Total CDLQI score**
Average wrist activity (g min^{-1})	**0.302**	0.414*	**0.257**	0.267	0.440*
Frequency-specific activity (mg min^{-1})	**0.257**	0.266	**0.196**	0.147	0.291
Subjective score	**0.491****	0.519**	**0.535****	0.443*	**0.573****
Pruritus	**0.468***	0.529**	**0.467***	0.472*	**0.580****
Sleep loss	**0.464***	0.462*	**0.540****	0.377*	**0.513****

** $p < 0.01$, * $p < 0.05$; 2-tailed.

Table 4.8 Correlations of Digitrac data with several components and the total Children's Dermatology Life Quality Index (CDLQI) score at the baseline of TCM trial. In particular, subjective scores reported by the parents correlated well with questions related to scratching and sleep loss and total CDLQI. Such a correlation could not be found using Di0gitrac data.

a)



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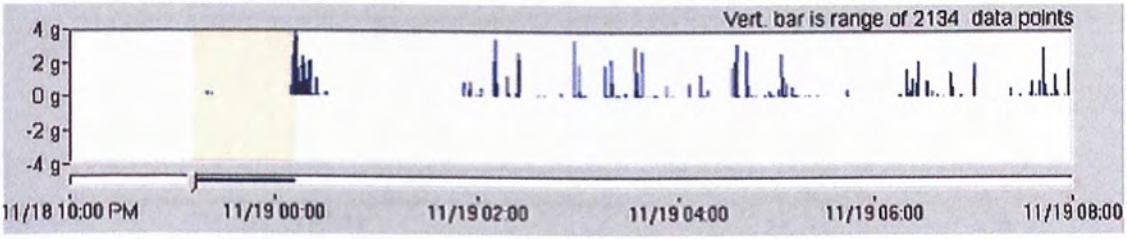


c)

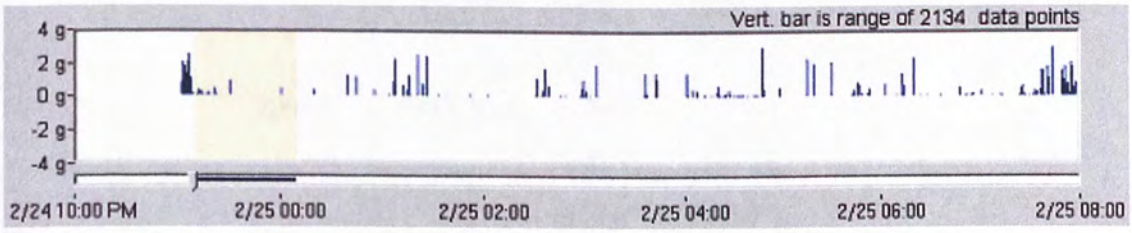


Figure 4.4 Visualization of wrist activities in early hours of sleep of a) a severe AD patient at the baseline of, b) the same patient at the end of TCM trial, and c) a normal healthy control of same age (6y) as reference.

a)

Mean (SEM) wrist activity (g min^{-1})	First hour	Second hour	Third hour	Average 0-3 hour
Baseline	121.2	107.0	112.0	113.4
	(17.2)	(16.0)	(19.0)	(12.2)
End of 12-week trial	89.6	72.5	66.6	76.2
	(11.7)	(10.1)	(8.1)	(8.1)
p	0.055	**0.043***	**0.012***	**0.001****

** $p < 0.01$, * $p < 0.05$; 2-tailed.

b)

Mean (SEM) Hz-specific wrist activity (mg min^{-1})	0-1 Hz	0-2 Hz	0-3Hz	Total 0-3 Hz
Baseline	286.7	225.2	177.5	689.4
	(36.9)	(27.0)	(21.9)	(84.5)
End of 12-week trial	157.5	140.6	112.1	410.3
	(20.9)	(17.2)	(13.9)	(50.0)
p	**0.001****	**0.003****	**0.004****	**0.002****

** $p < 0.01$, * $p < 0.05$; 2-tailed.

Table 4.9 Mean a) wrist activity for AD patients and controls (g min^{-1}) and b) frequency-specific wrist activity (mg min^{-1}) before and after TCM trial. Difference among 2 groups was significant for every single hour over 0-3 hour except the first hour.

Average wrist activity ($g\ min^{-1}$) (n=56)		First hour of sleep	Second hour of sleep	Third hour of sleep	Average 0-3 hours
SCORAD	**Pearson ρ**	**0.100**	**0.369****	**0.338***	**0.358****
	p	**0.462**	**0.005**	**0.011**	**0.007**
Objective SCORAD	**Pearson ρ**	**0.095**	**0.388****	**0.347****	**0.368***
	p	**0.484**	**0.003**	**0.009**	**0.005**
Extent	**Pearson ρ**	**0.056**	**0.434****	**0.396****	**0.391****
	p	**0.683**	**0.001**	**0.003**	**0.003**
Intensity	**Pearson ρ**	**0.107**	**0.363****	**0.321***	**0.350****
	p	**0.434**	**0.006**	**0.016**	**0.008**
Erythema	Pearson ρ	0.010	0.365**	0.292*	0.294*
	p	0.943	0.006	0.029	0.028
Edema / Papulation	Pearson ρ	0.060	.365**	0.343**	0.340*
	p	0.659	0.006	0.010	0.010
Oozing / Crusting	Pearson ρ	0.290*	0.132	0.200	0.280*
	p	0.030	0.334	0.140	0.036
Excoriation	Pearson ρ	0.091	0.200	0.309*	0.269*
	p	0.504	0.140	0.020	0.045
Lichenification	Pearson ρ	0.040	0.248	0.239	0.233
	p	0.770	0.065	0.077	0.084
Dryness	Pearson ρ	-0.010	0.334*	0.088	0.176
	p	0.942	0.012	0.520	0.194
Subjective score	**Pearson ρ**	**0.096**	**0.234**	**0.243**	**0.254**
	p	**0.483**	**0.083**	**0.071**	**0.058**
Pruritus	**Pearson ρ**	**0.075**	**0.255**	**0.236**	**0.251**
	p	**0.583**	**0.058**	**0.080**	**0.062**
Sleep loss	**Pearson ρ**	**0.105**	**0.197**	**0.228**	**0.237**
	p	**0.440**	**0.147**	**0.091**	**0.079**

** $p < 0.01$, * $p < 0.05$; 2-tailed.

Table 4.10 Correlations of average wrist activity ($g\ min^{-1}$) with SCORAD, objective SCORAD and related component scores using set of data from TCM trial. Objective wrist activity readings correlated well with objective clinical scores (SCORAD, objective SCORAD, extent and intensity), but not with subjective reports of pruritus and sleep loss.

Mean (SEM) plasma level (pg ml^{-1}) (n=28)	Baseline	End of trial	*p*
TARC	824	493	0.013*
	(189)	(101)	
CTACK	1424	1087	0.020*
	(136)	(66)	
BDNF	1798	1378	0.002**
	(177)	(146)	
Substance-P	93.6	72.9	0.002**
	(8.0)	(6.4)	

** $p < 0.01$, * $p < 0.05$; 2-tailed.

Table 4.11 Changes in laboratory markers before and after TCM clinical trial.

	n=56	BDNF	SP
Subjective Score	Pearson ρ	0.252	0.113
	p	0.061	0.407
Pruritus	Pearson ρ	0.248	0.097
	p	0.066	0.478
Sleep loss	Pearson ρ	0.235	0.117
	p	0.081	0.389
Average wrist activity (g min^{-1})	**Pearson ρ**	**0.878****	**0.897****
	p	**<0.00001**	**<0.00001**
Frequency-specific activity (mg min^{-1})	**Pearson ρ**	**0.789****	**0.801****
	p	**<0.00001**	**<0.00001**

** $p < 0.01$; 2-tailed.

Table 4.12 Correlations of pruritic markers, BDNF and substance P, with subjective symptom scores and Digitrac data.

4.3 Tacrolimus clinical trial

Three boys and four girls, with a median (range) age of 11.8 (5.3-18.4) years, participated in the study. Their demographic characteristics are given in table 4.13. The average amount of Tacrolimus used over the 2-week course by the patients was 19.5 gm. One of the patients reported itch sensation with topical application of Tacrolimus. Compliance to treatment was good and there was no report of oral antihistamine or topical CS usage during the study period for all patients.

Their median (interquartile range) objective SCORAD scores before and after treatment were 27.2 (24.8-36.7) and 23.9 (22.6-36.5), respectively ($p=0.248$). The overall SCORAD scores before and after treatment were 36.1 (32.8-45.7) and 29.4 (22.6-36.5), respectively ($p=0.059$; Figure 4.5). The total SCORAD was reduced in six patients (range: 8-36% reduction) and remained similar in 1 patient. No significant change in the area or intensity component of SCORAD was detected 14 days after treatment ($p=0.48$, $p=0.115$, respectively). The median (interquartile range) of pruritus and sleep disturbance component of the SCORAD reduced from 5.0 (5.0-6.5) and 4.0 (3.5-5.0) to 4.0 (2.0-5.0) and 3.0 (0.5-4.5); $p=0.033$ for pruritus, $p=0.268$ for sleep, respectively, after performing paired-samples t-tests. The median scratching activity during the first 3 hours of sleep, as documented by the Digitrac movement recorder, reduced from 115.0 (64.8-215.5) g min^{-1} at baseline (day 8), to 67.6 (47.8-152.0) g min^{-1} ($p=0.052$) on day 10, and 71.5 (51.0-118.0) g min^{-1} ($p=0.028$ by Wilcoxon signed rank test) at 2 weeks (day 22) (Figure 4.6). The daily symptom scores (n=6 pairs) reported each day separately by patients and parents for sleep disturbance agreed well with each other [intra class coefficient (ICC)

0.60-0.98]. These scores became significantly reduced two days following treatment (Figure 4.8; p=0.03 and 0.013, respectively). Only six pairs of data were obtained for the daily scores because one young girl was unable to give reliable scores for her symptom of sleep disturbance.

For laboratory findings, levels of TARC and CTACK (units in mean (SEM)) decreased significantly from 1135(462) to 514 (345) and from 1605 (283) to 1375(270) pg ml^{-1}, respectively (p=0.018) (Table 4.14). The levels of pruritic markers BDNF and SP, however, did not show any statistical significance.

The small pilot study have successfully demonstrated that scratching movements, objectively measured with the Digitrac, and subjective score of itch and sleep disturbance can be separately recorded to assess therapeutic effects of a treatment. Tacrolimus appeared to have faster effects on subjective than objective symptomatology. The study also identified that battery life span (usually 14 days) is not a limiting factor for serial scratching measurement but the memory capacity of Digitrac (10-hour overnight recording) is. In order to monitor the serial effects of a tested medication, the data have to be downloaded daily.

Due to the small sample size and the wide age range, specific comments as to why the extent and subjective symptoms are not improved were not able to be made. Although AD-associated chemokines showed a significantly reduced level, SCORAD, BDNF and SP did not. It might be that the change in disease extent and intensity as measured by the SCORAD and laboratory parameters were insensitive in a short test period of two weeks.

Measurement of subjective symptoms is notoriously unreliable, especially with the small sample size. The rapid onset of action for Tacrolimus described in this open-label study should be confirmed with a double-blind randomized controlled trial using our dual approach, combining objective and subjective tools. More importantly, we propose that all novel topical and systemic therapy should be evaluated with such a combined approach. Despite these, individual patients might significantly benefit from the use of this medication. Tacrolimus appeared to promptly improve sleep disturbance within days but did not reduce scratching at two weeks following its use. A large randomized placebo-control study is underway to evaluate the rate and extent of itch reduction of Tacrolimus in children with AD.

Tacrolimus has played an important part in the treatment of paediatric AD since its introduction in 2001, yet the recently recommended black box warning from the U.S. Food and Drug Administration (FDA) has led to a re-examination of its role, particularly in paediatric patients (US Food and Drug Administration, 2005). What worried the scientists was the possible elevation of blood level for patients who received sustained medication of Tacrolimus, which might lead to adverse effects, if any, after prolonged application over large surfaces of skin (Berger *et al.*, 2006). Increased rate of lymphoma was shown by dermal toxicity and carcinogenicity studies in animal models (Niwa *et al.*, 2003), yet the doses far exceeded values achieved by topical use in humans. Therefore, as prolonged overuse of topical CS to certain anatomical areas including the face has a particular potential for adverse events like skin atrophy, striae, systemic absorption and adrenal suppression (Berger *et al.*, 2006), second-line treatments of AD like Tacrolimus

are still of great necessity, yet should be used only if other therapies are ineffective or inappropriate and the course of application should be short-term and intermittent. Nevertheless, strict monitoring clinical dose of both medications is necessary.

Subject	Gender	Age	Run-in SCORAD	Baseline SCORAD	Treatment SCORAD	SCORAD improvement?	Baseline serum Total IgE (kIU/ml)	Post-treatment Total IgE (kIU/ml)
1	F	13.20	36.9	30.6	24.3	YES	334	393
2	M	11.79	35.5	32.4	29.4	YES	1264	906
3	F	14.07	28.1	33.2	24.7	YES	611	781
4	M	11.63	43.5	38.7	24.9	YES	135	150
5	F	7.96	48.0	52.7	48.6	YES	3219	3335
6	M	18.39	30.7	36.1	42.2	NO	2292	2124
7	F	5.32	30.5	65.3	56.8	YES	7479	10261

Table 4.13 Demographic characteristics of patients enrolled into Tacrolimus pilot study.

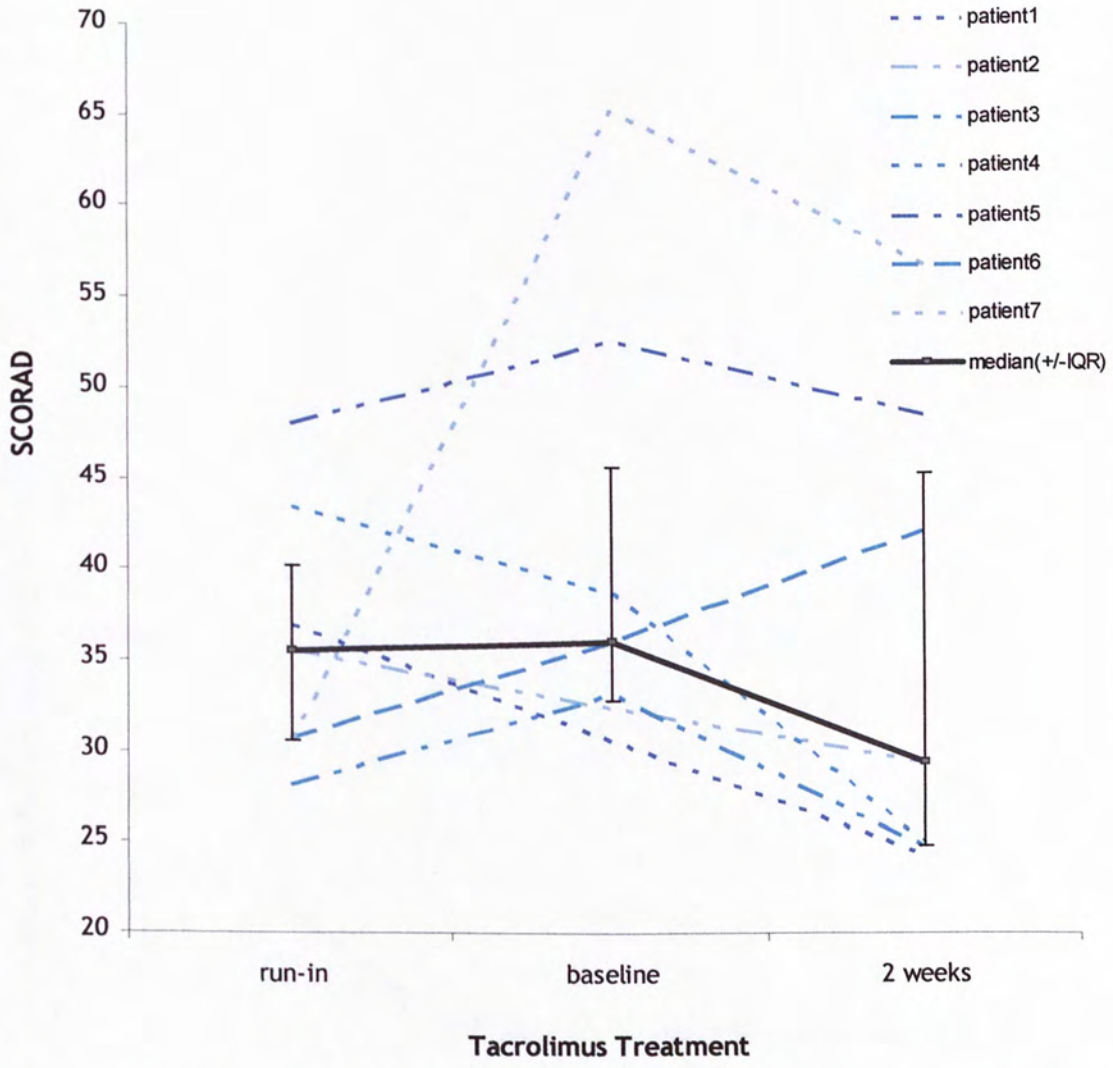


Figure 4.5 Changes in total SCORAD scores during the 2-week study period of 7 patients in Tacrolimus pilot study. Treatment commenced on day 8 following a one week run-in and completed on day 21.

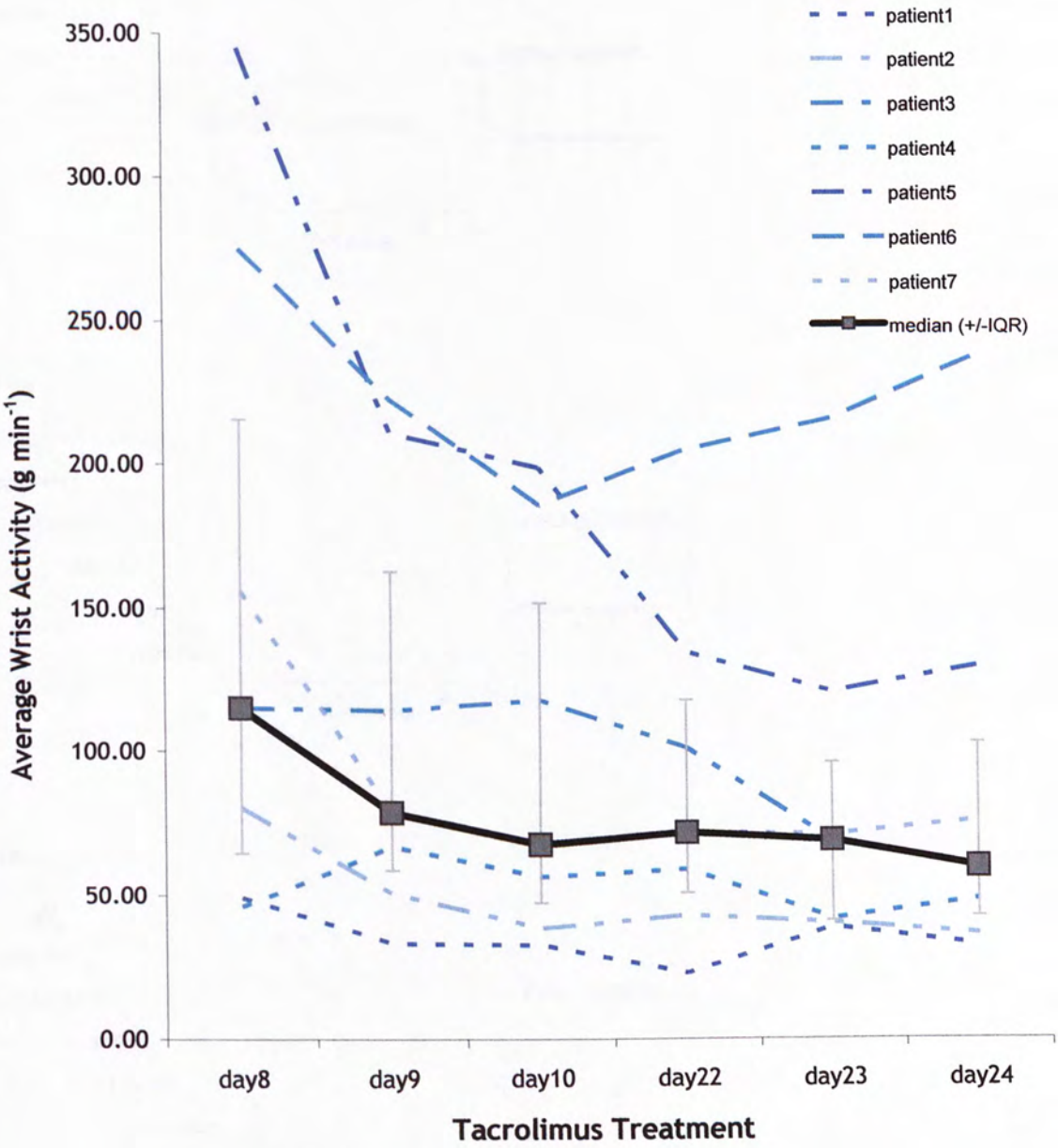
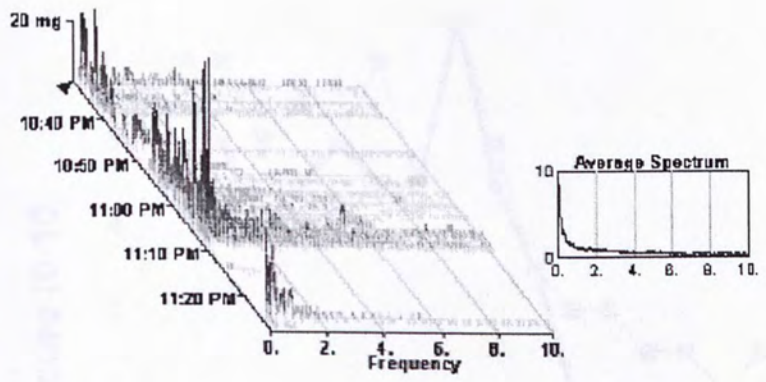


Figure 4.6 Average wrist activity (g min^{-1}) for the first 3 hours of sleep of the patients during Tacrolimus treatment.

a)



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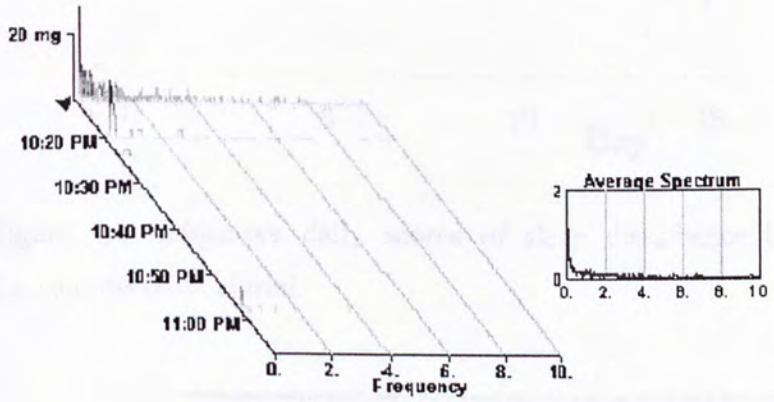

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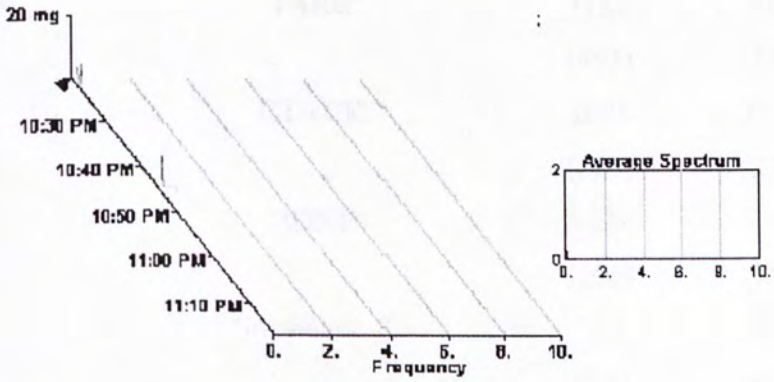


Figure 4.7 Frequency spectra of wrist activities in the early hours of sleep of (a) a severe AD patient at the baseline of, (b) the same patient at the end of Tacrolimus trial with (c) a normal subject of same age as reference. Most activities occur at 0 - 2 Hz.

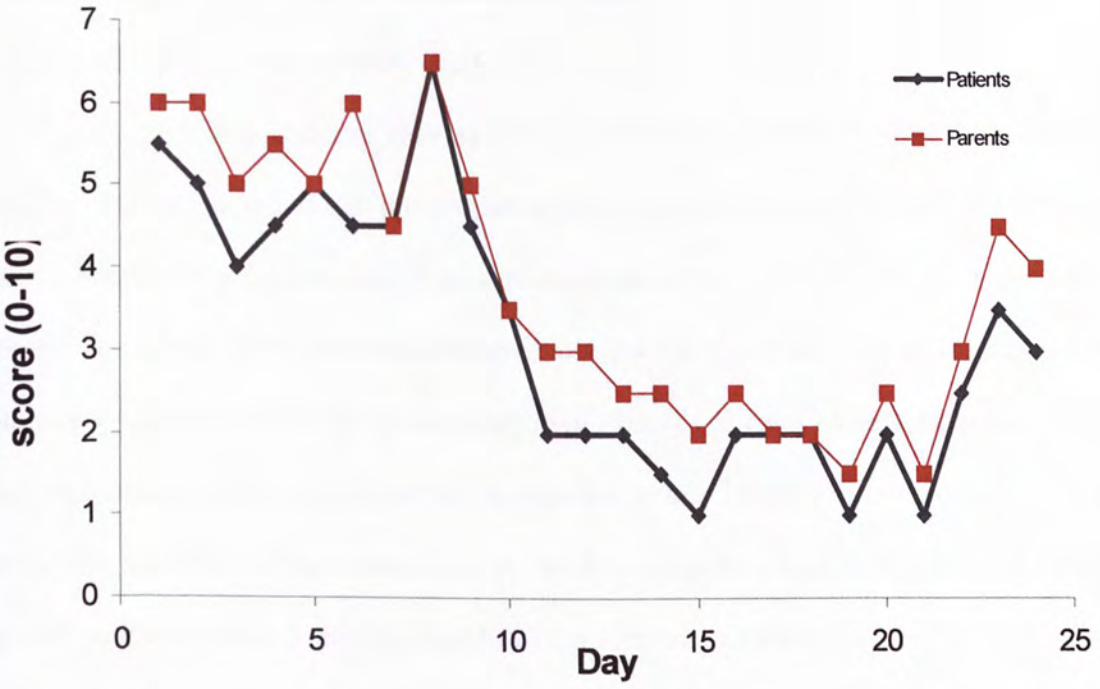


Figure 4.8 Subjective daily scores of sleep disturbance by parents and patients in Tacrolimus clinical trial.

Mean (SEM) plasma level (pg ml^{-1}) (n=7)	Baseline	End of trial	*p*
TARC	1135	514	0.018*
	(462)	(345)	
CTACK	1605	1375	0.018*
	(283)	(270)	
BDNF	1621	1502	NS
	(206)	(156)	
Substance-P	71.4	70.9	NS
	(9.6)	(9.6)	

* $p < 0.05$, 2-tailed. ; NS, not significant.

Table 4.14 Changes in laboratory markers before and after Tacrolimus clinical trial.

4.4 Application of Digitrac in other areas of study

4.4.1 Pemphigoid gestationis case study

Pemphigoid gestationis (PG) is a rare, yet intensely pruritic bullous dermatologic disease of pregnancy and the postpartum period. It usually starts around the second or third trimester of pregnancy, with an estimated incidence of one in 50,000 pregnancies (Wong and Chua, 2002;mbros-Rudolph *et al.*, 2006). The exact incidence among the Chinese is unknown. PG may be associated with Graves disease, hydatidiform moles and choriocarcinomas (Shornick, 1993;Chimanovitch *et al.*, 1999). The patient in this case study had a history of chorioangioma in her first pregnancy and Graves disease in the second pregnancy and the history matched with such associations.

In PG, chemoattraction of eosinophils and their subsequent degranulation is followed by the action of proteolytic enzymes, which dissolve the bond between epidermis and dermis. T-helper cells have been implicated in the very early stages of autoimmune response and may exercise a broad influence in subepidermal blister formation in this disease (Fabbri *et al.*, 2003).

The onset of PG is abrupt with intense pruritic urticarial lesions on the trunk, which usually spared the face, mucosal membranes, palms, and soles (Engineer *et al.*, 2000). Lesions begin usually on the abdomen adjacent to the umbilicus, though atypical presentations like palm and soles are observed. The clinical course is variable; many experience spontaneous resolution during the later part of gestation, but some of which may have flare again during the time of delivery. The patient in this case study had a transient recurrence in the immediate postpartum period. Recurrence in majority of patients during subsequent pregnancies has been reported, but there was no evidence that

the disease will be more severe in subsequent pregnancies. The patient must be monitored closely in subsequent pregnancies.

Nocturnal itch pattern of the patient documented with the Digitrac wrist monitor showed intensive scratching movements (Figure 4.9a), with an average value of 181.0 ± 43.5 (mean ± SEM) g min^{-1} for the first three hours, which was higher than the average value of 113.4 ± 10.2 g min^{-1} for the group of 43 eczema subjects in validation study. Most wrist activities are slow movements at 0 to 2 Hz (Figure 4.10). The readings indicated the large extent of pruritus and nocturnal sleep disturbance of this patient. The wrist activities of a normal person and that of a patient with severe eczema were also shown as reference (Figure 4.9b and 4.9c). The frequency range of activity was in similar to the scratching activities at 0 to 3 Hz in eczema subjects. Basing on these results and comparisons of pruritus patterns of long-term AD patients, the intense scratching and discomfort experienced by patients with pemphigoid gestationis must not be underestimated.

The patient was symptomatically treated with antihistamines (chlorpheniramine meleate 5 mg) and topical CS (flucinolone acetonide 0.025%) and her skin symptoms resolved over a period of 2 weeks. She was also treated as having Graves disease with propylthiouracil (50 mg, twice daily) for two months. Subsequently, she gave birth at thirty-nine weeks three days of gestation by normal delivery to a healthy baby girl who weighed 2.50 kg with Apgar scores of 9 and 10 at one minute and five 5 minutes, respectively. She had recurrence of blistering lesions on her hands and trunk four days postpartum but the lesions subsided on further treatment with topical CS.

This short report served to alert clinicians, especially obstetricians and general practitioners, of the importance of considering the various differential diagnoses in a pregnant woman with bullous generalized itch. A dermatologist, an obstetrician and a paediatrician must coordinate care for patients with pemphigoid gestationis. Although PG is an uncommon disease in pregnancy among the Chinese, and differential diagnoses may include other bullous conditions, this diagnosis can be readily confirmed with histopathological studies. This case also illustrated that the scratching and discomfort experienced by patients with pemphigoid gestationis were intense and must not be underestimated. As itch is difficult to be documented objectively, the Digitrac monitor could help determine on its management, such as the choice of various sedating antihistamines or initiation of systemic CS if the itch is severe. Digitrac might also be helpful in monitoring its intensity and frequency before and following treatments.

4.4.2 T-cell lymphoma case study

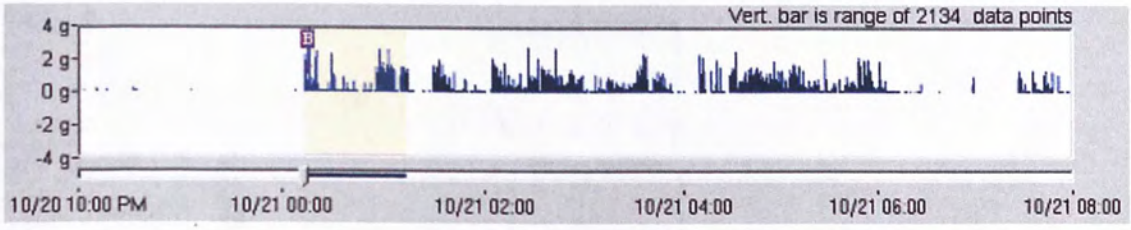
Previous clinical trials stressed the wrist activities between 0 and 3 Hz for the first three hours of sleep as good indicator of eczema severity, nocturnal itch and sleep disturbance in children. It was also found that wrist activities in this patient occurred primarily in the first few hours of sleep at baseline before receiving chemotherapy (Figure 4.11). The intensity of these movements was even higher than that in patients with severe eczema, with an average value of 223.6 ± 97.6 (mean ± SEM) g min^{-1} for the first three hours versus 113.4 ± 10.2 g min^{-1} for the group of 43 eczema subjects, while decreases to 69.2 ± 9.9 g min^{-1} at Day 6. The frequency pattern also showed a drastic decrease after chemotherapy, with a 4-year-old control as reference (Figure 4.12).

Interestingly, the itch was not relieved with antihistamines but by chemotherapeutic treatment of the underlying malignancy, implying the fact that mechanisms other than histamine may be involved in the pathophysiology of pruritus in this patient. Most wrist activities were very slow ones, with a strong emphasis at 0 to 1 Hz region only. The frequency bandwidth is lower than that of AD patients.

It is a well-known paradigm in dermatology as well as in oncology that malignancies, among other differential diagnoses, must be considered in any patients with new onset of generalized itch (Kleyn *et al.*, 2006). This consideration is especially important if the patient does not have any apparent rash to account for the symptom. The other differential diagnoses of generalized itch without a primary dermatological etiology includes iron deficiency anaemia, thyroid disease, hepatic failure, drugs, and polycythaemia rubra vera. Delayed or missed diagnosis in these conditions may not cause immediate mortality. Conversely, delays in diagnosing malignancy such as lymphoma may result in widespread dissemination and irreversibly loss of time for treatment.

This second case study served to alert dermatologists, oncologists, paediatricians and general practitioners, of the importance of considering the various differential diagnoses in a previously well child with an unexplained generalized itch. This case also further challenged the anti-pruritic effect of antihistamines in this case, which the controversy is the same as for AD patients. The application of Digitrac illustrated further the potential possibilities of using the device in differential diagnosis as well as quantifying itch before and following treatment in various conditions associated with itching and scratching with different drugs, and in all patients with chronic pruritus, not only confined in AD.

a)



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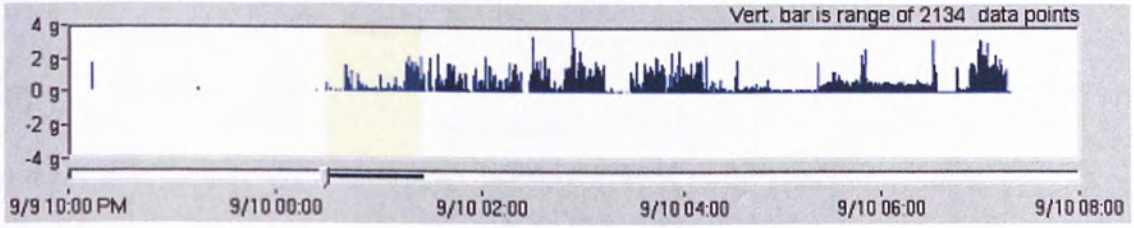

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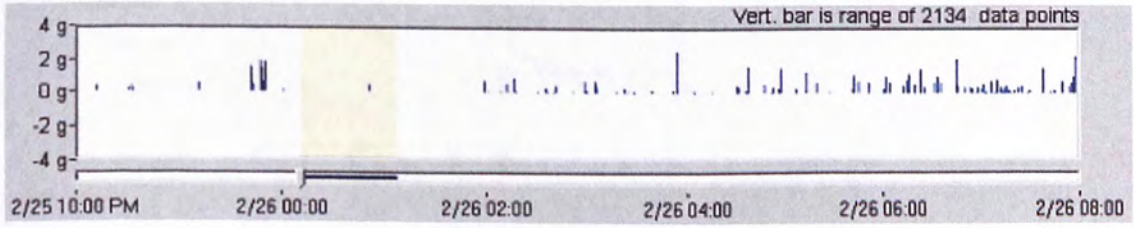
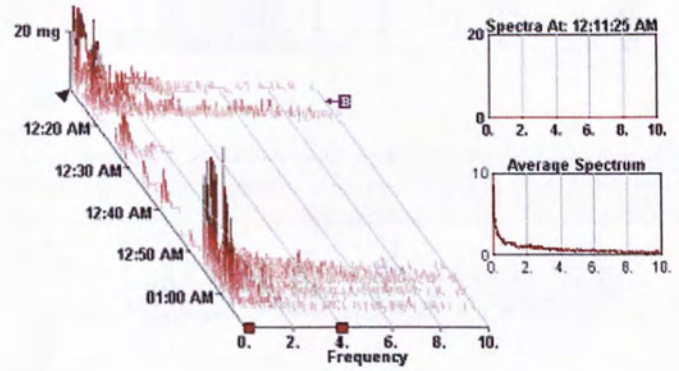


Figure 4.9 Average wrist activities of (a) the woman with pemphigoid gestationis, (b) a 21-year-old male with severe atopic dermatitis, and (c) a normal 21-year-old female as reference.

a)



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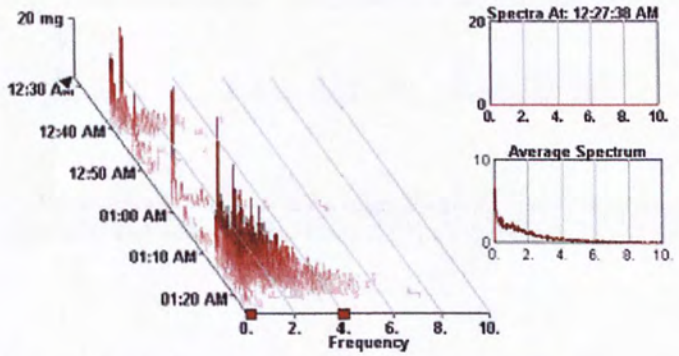

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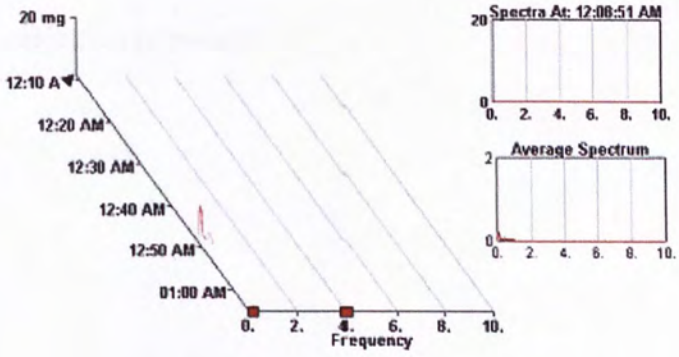


Figure 4.10 Frequency spectra in the early hours of sleep of (a) the patient suffering from pemphigoid gestationis, (b) a 21-year-old male with severe atopic dermatitis, and (c) a normal 21-year-old female as reference. Note that most activities occur at 0 - 2 Hz.

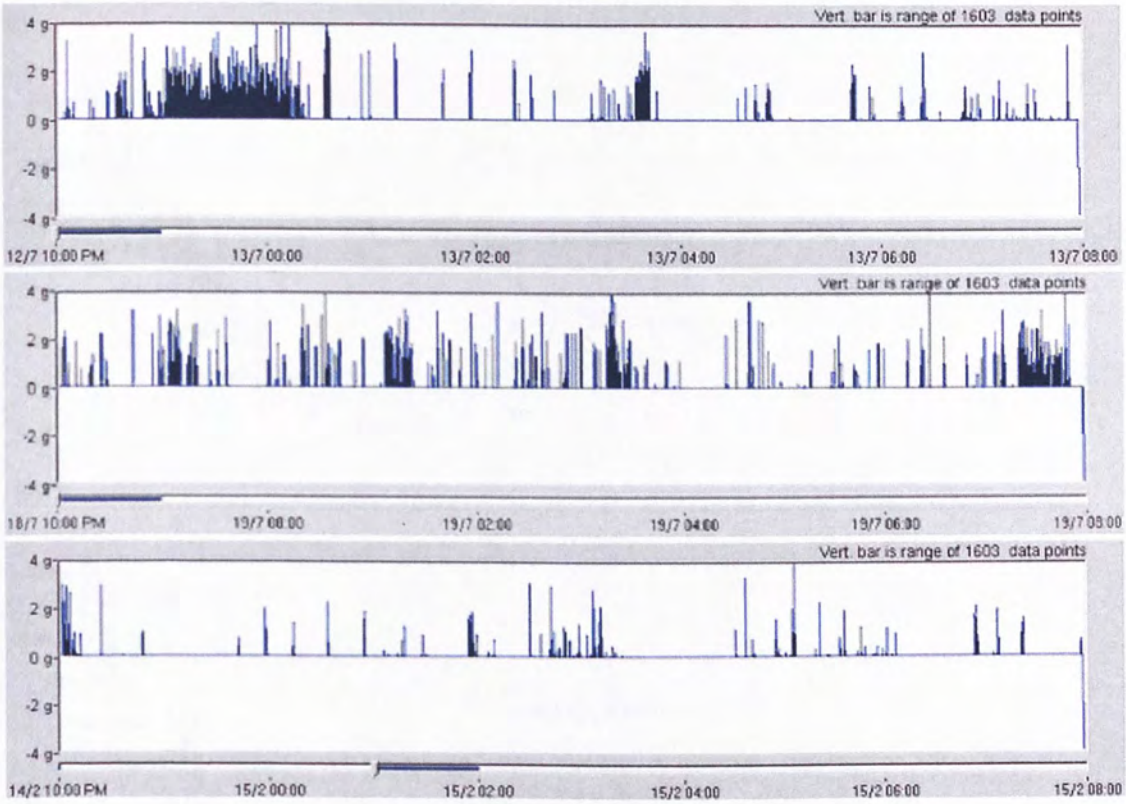
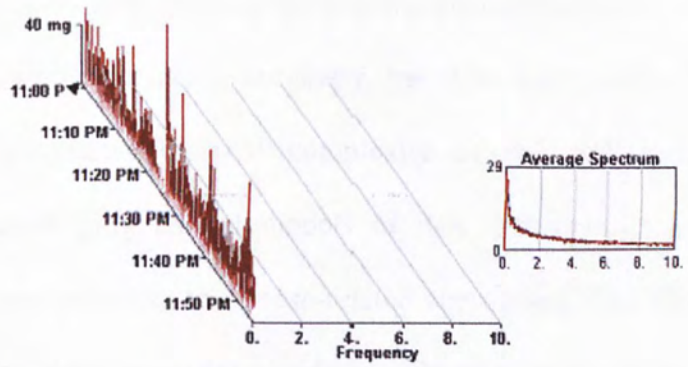


Figure 4.11 Average wrist activities of the patient suffering from T-cell lymphoma at (from up to down) (a) baseline, (b) on day 6 following chemotherapy, and (c) a normal subject as reference.

a)



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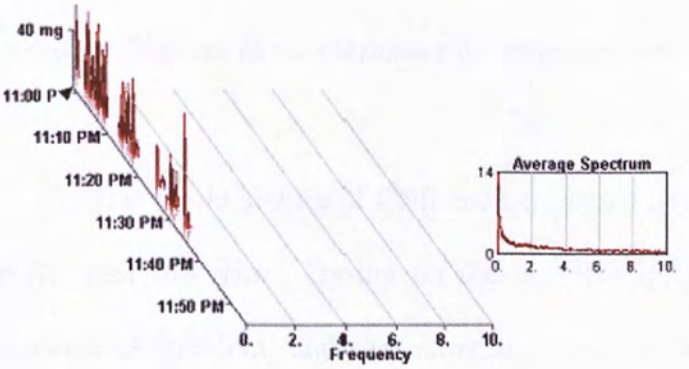

c)

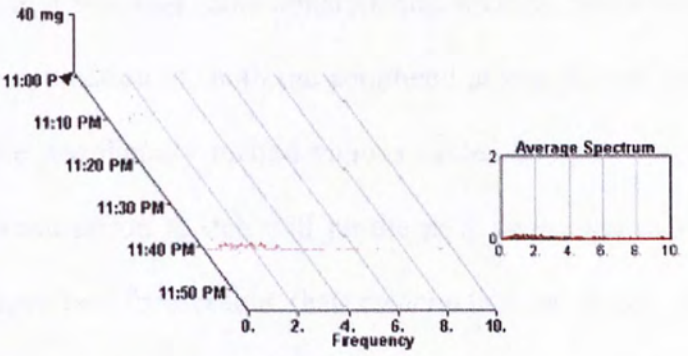

Figure 4.12 Wrist activities and frequency spectra in the early hours of sleep at (a) baseline, (b) on day 6 following chemotherapy, and (c) a normal subject as reference of the patient with lymphoma. Note that most activities occur at 0-1 Hz.

Chapter 5

Further Discussions and Conclusion

The research on pruritus management and development of a more effective and tailored regime is necessary, yet slow due to the complexity of neurophysiological and neuroimmunological complexity of itch pathophysiology (Paus *et al.*, 2006a). In developing animal models of itch, direction has already been changing from acute injection ones to disease-related approaches. The difficulties in obtaining a methodology for objective, reproducible quantification of pruritus is also critical. While the majority of simple pruritus researches still treat visual analogue scales as the gold standard, we aim to introduce Digitrac as an alternative for objective behavioral assessments.

The whole picture of CNS and peripheral nervous system has been explored a lot in the past few years. Basing on the fact that sleep disorder is still one of the most tremendous problem, and that currently there are no accurate remedies to deal with, antihistamines will remain as a main basis, especially basing on their sedative effects. Yet it becomes more apparent and effective that a treatment regime basing on a targeting combination of both the peripheral production of inflammation-induced itch signals and the peripherally incited vicious cycles that perpetuate itch and cause spinal and central sensitization to itch will be the next generation of anti-pruritic treatment strategy. It is therefore foreseeable that combination of drugs that counteract chronic central itch sensitization with peripheral anti-inflammatory agents is a particularly sensible approach beyond the antihistamine horizon.

To conclude for the whole study, it has fulfilled its primary objective to look for alternatives in objective assessments of pruritus. It is hoped that measurement of wrist movements served the purpose as a better alternative for clinicians and researchers to quantify important clinical data on pruritus and sleep loss in an objective, home-based and convenient manner.

To further apply the device in research, continuous measurements should be performed. In the validation part, only one single night was measured. Continuous measurements of wrist activities might throw more lights on any differences between monitoring over weekdays versus weekend, or examination versus usual school days, or school days versus long vacation. Moreover, usage of the device might not be confined in AD only, but also be applicable in other diseases with movement disorders, e.g. Parkinson's tremor.

Concerning the use of the device in atopic dermatitis research, emphasis should be focused on the early hours of sleep at low frequency range. 1-2 days of recording appears to be sufficient for meaningful data capturing, though consecutive measurement would be suggested. Moreover, the device should be used alongside with other outcome measures, e.g. CDLQI in order to take a dual approach to consider both objective and subjective outcome measures simultaneously.

For localized lesions of AD, usage of topical corticosteroids remains its key role to sooth the acute worsened situation. In fact, a short courses steroid with appropriate

potency is the cheapest, safest, and the most effective medication to apply for early stages of skin inflammation. However, one should never neglect the growing importance of alternatives including traditional herbal medication, in particular, Chinese herbs. Application of tacrolimus possesses no burning sensation. For an ethical scientist and practitioner, safety should always come first. Last but not least, AD skin lesions are often associated with secondary infection of *Staphylococcus aureus.* Treatment of antibiotic is still necessary

It is foreseeable that more and more new treatment formulae will be out soon. For the researchers, possible negative impacts of all these treatments have to be informed clearly to the patients and subjects. Last but not least, patience and care towards them is a must for all of the researchers in putting effort to work against the diseases that trouble millions for long.

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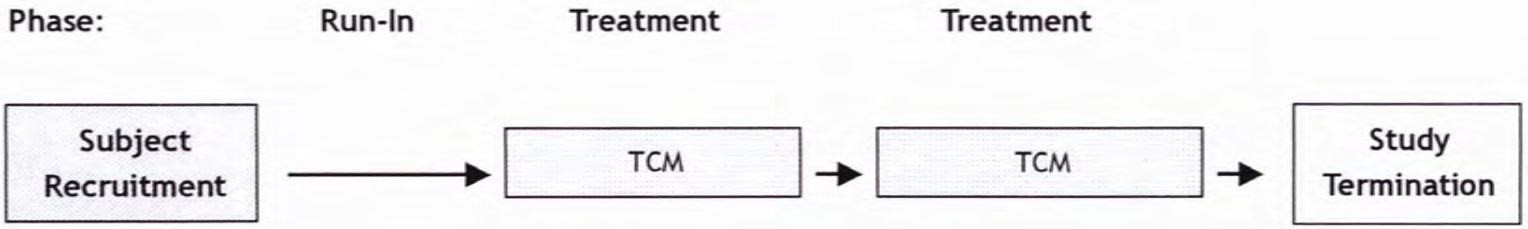
Appendix I. Protocol used for traditional herbal medication clinical trial

Schematic Representation of Traditional Chinese Medication clinical trial study protocol



Total 28 patients
with objective SCORAD ≥ 15

	4-w		6-w			6-w				
Week:	**0**	**1**		**2**		**3**		**4**		**5**
Follow-up Visit*:	↑	↑				↑				↑
Written Consent:		√								
Study Outcomes:										
CDLQI score		√								
SCORAD score		√				√				√
DigiTrac		√								√
AD markers (TARC / CTACK)		√								√
Pruritic markers (Substance P / BDNF)		√								√
Others (CBC, IgE, etc)		√								√
Diary / Adverse Events:	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Relief Drugs*:										
Drug Compliance*:		√				√				√

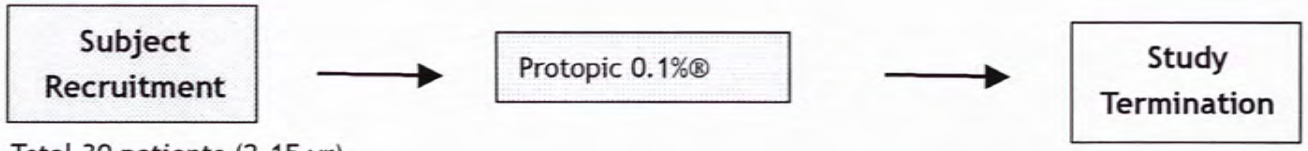
***Note:**

- Dietary intake *and* emollients for AD must **remain unchanged** during the entire study period.
- Use of relief drugs (including sedating oral anti-histamines and topical corticosteroids) are allowed throughout the study for ethical reasons and would be recorded whenever patients violate the rule.

Appendix II. Protocol used for tacrolimus clinical trial

Schematic Representation of Tacrolimus Clinical Trial Study Protocol

Phase: Run-In Treatment



Total 30 patients (2-15 yr)
with objective SCORAD ≥ 15

Week:	**0**	**1**		**2**		**3**		
Follow-up Visit*:	↑	↑				↑		
Written Consent:	√							
Study Outcomes:								
SCORAD score	√	√				√		
DigiTrac		√	√	√		√	√	√
AD markers								
(TRAC / CTACK)		√				√		
Pruritic markers								
(Substance P / BDNF)		√				√		
Others (CBC, IgE, etc)		√				√		
Diary / Adverse Events*:		√	√	√	√	√	√	√
Relief Drugs*:								
Drug Compliance*:		√				√		

***Note:**

- Dietary intake *and* emollients for AD must **remain unchanged** during the entire study period.
- Use of relief drugs (including sedating oral anti-histamines and topical corticosteroids) are **NOT** allowed throughout the study for ethical reasons and would be recorded whenever patients violate the rule.

Appendix III. Written consent (in Chinese) used in traditional herbal medication clinical trial.

中药對中國藉濕疹病童的療效及安全性

研究背景

濕疹是一種由致敏原引起的常見皮膚炎病。濕疹和哮喘等敏感病爲香港兒童最常患上的慢性疾病之一，約九成濕疹病童的發病年齡在五歲以下。治療濕疹以潤膚及外敷药爲主，近年來中药開始受到重視，但其對濕疹之效果及副作用，卻未被深入研究。

研究目的：中醫药對中國藉濕疹病童的療效及安全性。

是項研究將於門診部徵求中國籍濕疹病童。並根據歐洲工作小組提出的 SCORAD 指數及其它濕疹指數，作臨床評定濕疹的程度。再者，中药對身體的生理功能如肝、腎及血的影響，也會小心跟進。

項研究將包括臨床指標及實驗室指標。負責這研究的敏感科醫生，會先爲貴子弟抽取約 5 毫升血液以化驗免疫球蛋白 E 的濃度，嗜伊紅細胞陽離子蛋白含量及其他發炎指標的濃度；而兒童皮膚科醫生則會詳細評估貴子弟濕疹的嚴重程度。貴子弟將會接受中药共 12 週，如您或您的子女對當中成份產生敏感，請停止服用。請將現在或以往的醫療記錄及對那些物質有過敏的情況告訴醫生（或醫護人員）。通常中药不會干擾您或您的子女所服用的其他藥物。但請將您所服食或準備服食的藥物（包括非經處方得到的藥物）告訴醫生。每日按醫生的處方服食，是否跟食物一起服用均可。爲了可以控制濕疹，持續依照醫生處方服用十分重要。如少服食一次，只要繼續依照正常服藥時間便可。任何藥物都可能導致一些非預定的反應或稱副作用。大部份病人都能接受本藥。副作用通常都屬輕微。如果出現不尋常徵狀或徵狀持續或惡化，請告知醫生。包裝盒及包裝上印有藥物有效日期，切勿使用過期藥物。放於室溫乾爽處。小心存放，避免被小童誤服。

研究人員亦會於期間給予貴子弟Digitrac手部運動記錄器，並對解釋使用方法。Digitrac 手部運動記錄器本身用於記錄運動員運動情況，並協助其改善表現；今次乃首次用於醫療方面，並在是次研究中，記錄病童於入睡前的手部抓癢三維擺動幅度，以進行數據分析，客觀及無創地評估兒童在療程其間，其睡眠質素及痕癢狀況。

同意書

本人爲病人_____之父母，現同意本人之子女參予這項研究。本人明白並接受上述研究之要求，在有需要時也可向負責此研究的醫生詢問子女各項測試的結果。本人也明白可以隨時退出此項研究而不需作出任何解釋，而本人的子女將繼續享有和現時同樣質素的治療。病人資料只會用於此項研究，不會公開。

父/母姓名：_____　　父/母簽署：_____

父/母之身份証號碼：_____　　簽署日期：_____

見証人姓名：_____　　見証人簽署：_____

Appendix IV. Written consent (in Chinese) used in tacrolimus clinical trial.

以 Digitrac 運動記錄器評估 Tacrolimus (Protopic®) 無類固醇外敷藥對中國藉濕疹病童的痕癢及睡眠療效

研究背景

濕疹是一種由致敏原引起的常見皮膚敏感病，爲香港兒童最常患上的慢性敏感疾病之一。很多患上濕疹的兒童到晚間會因爲痕癢，難以入睡，導致日間缺乏精神，或嚴重影響課堂及各類活動的表現；又因於睡前不斷抓癢、以致弄損皮膚，影響濕疹復原速度。治療濕疹以潤膚及外敷類固醇葯爲主，近年市場出現不含類固醇的藥膏，惟其成效及對免疫系統之影響仍有待仔細研究。

研究目的

探討Tacrolimus(Protopic®)無類固醇外敷藥對中度至嚴重濕疹病童之療效及對免疫系統之影響，並同時使用Digitrac手部運動記錄器，無創地觀察及研究中國藉濕疹病童在治療過程中睡眠質素及痕癢情況。

是項研究將維時約四星期。首先兒科醫生及研究人員會於門診部徵求中國籍濕疹病童，並根據歐洲工作小組提出的SCORAD指數及其它濕疹指數，作爲臨床評定濕疹的程度，並仔細解釋整個研究流程。

兒科醫生會於期間將藥物給予家長，並詳細解釋其藥性，潛在副作用，使用方法及劑量。若有任何不良反應，醫生會即時停止藥物臨床測試。由於藥物並不納入在醫管局標準藥物名冊內，病人如有興趣於測試後繼續使用藥物，則需出示醫生紙自行購買。

研究人員亦會於期間給予貴子弟Digitrac手部運動記錄器，並對解釋使用方法。Digitrac手部運動記錄器本身用於記錄運動員運動情況，並協助其改善表現；今次乃首次用於醫療方面，並在是次研究中，記錄病童於入睡前的手部抓癢三維擺動幅度，以進行數據分析，客觀及無創地評估兒童在療程其間，其睡眠質素及痕癢狀況。

是項研究將包括臨床及實驗室指標。貴子弟將會定期在門診隨訪，期間亦會配合及進行其他臨床及血液檢驗，以仔細評估病情及治療效果。家長及病童亦會獲發一張睡眠狀況日記，作記錄小朋友每晚之睡眠狀況，以跟進藥物療效。

有關病人的個人資料及研究數據，除相關之研究人員外，將嚴守秘密，以保障個人私隱。

同意書

本人爲病人_____之父母，現同意本人之子女參予這項研究。本人明白並接受上述研究之要求，在有需要時也可向負責此研究的醫生詢問子女各項測試的結果。本人也明白可以隨時退出此項研究而不需作出任何解釋，而本人的子女將繼續享有和現時同樣質素的治療。

父/母姓名：_____　　父/母簽署：_____

父/母之身份証號碼：_____　　簽署日期：_____

見証人姓名：_____　　見証人簽署：_____